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VITAMIN D AND PRO-VITAMIN D CONTENT OF THE AVOCADO[1]

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Vitamin D has been reported as a constituent of the avocado by Weatherby (1930) who, from a series of tests with rats, concluded that the avocado contains liberal quantities of vitamin D. Bacharach and Smith (1938) tested avocado oil and stated that although avocado oil did actually contain some vitamin D, the potency was low. Robbins and Bilger (1934) prepared purified sterols from avocado oil and assayed both the oil and the sterols prepared from it before and after irradiation. They found the irradiated only to be antirachitic, while the nonirradiated fractions had no antirachitic effect at all. Rosenberg (1942) states: "Vitamin D occurs in nature only in small amounts. The living plant tissue and fresh green vegetables contain no detectable amount of this vitamin. The occurrence of very small amounts of vitamin D in some species however is possible, due to the presence of significant amounts of pro-vitamin D." Since the avocado now is being used increasingly in the human diet, and in view of the contradicting evidence, this study was made to throw more light on the question by determining the vitamin D and pro-vitamin D content of the avocado.[2]

EXPERIMENTAL PROCEDURE

Preparation of Avocado Oil: The edible portion of the avocado was blended in the Waring blender to disintegrate the tissue and facilitate the extraction of the oil. Anhydrous sodium sulfate (purified grade) equal in weight to that of the avocado was mixed with the blended pulp and the oil was extracted by shaking the mixture in a five-liter, round-bottom flask with diethyl ether. The ether solution was decanted through a Büchner filter and the extraction repeated until the last extract was colorless and showed no evidence of the presence of sterol. The extracts were combined and the ether removed by distillation, the last part of it *in vacuo*. Part of the oil was used as such for the bio-assay of vitamin D and another part was used for the preparation of the unsaponifiable fraction.

Preparation of Unsaponifiable Fraction: Two hundred grams of avocado oil prepared above was stirred for one hour with 200 ml. of ethyl alcohol and 80 ml. of 50 per cent aqueous KOH. Saponification was completed by boiling the solution under reflux for eight hours on a water bath. The saponified mixture was diluted with 160 ml. of water and transferred to a liquid extractor and extracted continuously with petroleum ether [B.P. 30-60°C.(86-140°F.)]. The extraction was complete at the

[1] Supported by a grant from Avocado Growers of California.

[2] The Fuerte variety of avocado was used in these experiments.

end of 36 hours as shown by a lack of color in the extract and a negative test for phyto sterols (Lieberman reaction). The petroleum ether was removed by distillation, the last traces by use of vacuum. Part of the unsaponifiable fraction was taken up in corn oil for bio-assay; the other part was dissolved in diethyl ether and irradiated with a cold quartz lamp to activate any pro-vitamin D present. The condition of irradiation was such that ergosterol irradiated under identical conditions would give about 10 million U.S.P. units of vitamin D per gram of ergosterol. After irradiation the ether was removed and the residue dissolved in corn oil for bio-assay.

Bio-Assay of Avocado Oil, Unsaponifiable Residue, and Irradiated Unsaponifiable Residue: The method of the United States Pharmacopoeia, 11th decennial revision, 1939 supplement, pages 136-138, was used. The rats were fed the supplements shown (Table 1), which also includes the average line-test evaluation of each group.

TABLE 1

Results of Bio-Assay

Group [1]	Daily dose of supplement	Degree of healing
	Assay No. 1	
1	Negative control	0
2	Positive control; 0.6 U.S.P. units of vitamin D	1.5
3	0.2 ml. of fresh avocado oil	0
4	0.00482 gram of unsaponifiable fraction in 0.1 ml. of corn oil	0
5	0.000172 gram of irradiated unsaponifiable fraction in corn oil	0
	Assay No. 2	
1	Negative control	0
2	Positive control; 0.607 U.S.P. units of vitamin D	1.7
3	0.0265 gram of unsaponifiable fraction in 0.1 ml. of corn oil	0
4	0.00158 gram of irradiated unsaponifiable fraction in 0.1 ml. of corn oil	0

[1] There were seven rats in each group.

Colorimetric Tests for Vitamin D and Pro-Vitamin D: The unsaponifiable fraction and the irradiated unsaponifiable fraction were tested by four colorimetric qualitative tests for vitamin D and pro-vitamin D. The reversed Salkowski test was negative in both instances for vitamin D and pro-vitamin D, but did indicate the presence of phytosterols. The Lieberman and the Lieberman-Burchard tests were negative for vitamin D and the pro-vitamin, but indicated sterols to be present. The Tortelli-Jaffe

test was slightly positive for vitamin D or the pro-vitamin D in the unirradiated unsaponifiable fraction, but in the irradiated unsaponifiable fraction the test was negative, indicating that the substance responsible for the test had been destroyed by irradiation.

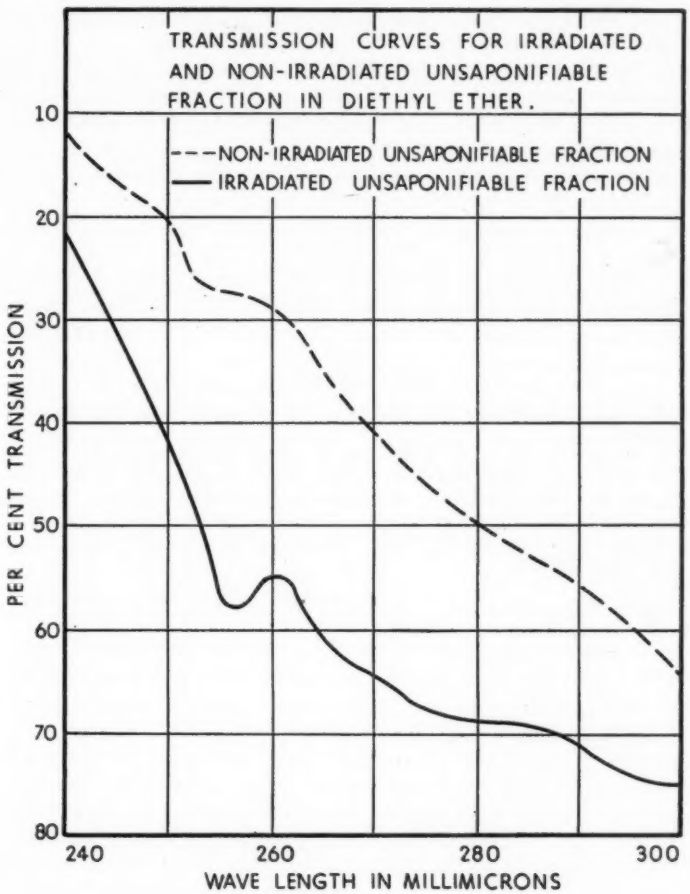

FIG. 1.

Spectrophotometric Method for Determination of Vitamin D: The unsaponifiable fraction and the irradiated unsaponifiable fraction were dissolved in diethyl ether, and the Spectral Transmission curves in the region from 240 to 300 millimicrons were obtained by the use of a Beckman Quartz spectrophotometer and are shown (Fig. 1).

DISCUSSION

The rat bio-assay experiments indicate that the vitamin D content of the avocado is very low. The oil, when fed at a level of 0.2 ml. per day,

gave an average degree of healing of zero (Table 1). The unsaponifiable fraction of the avocado oil was assayed for vitamin D at different levels, one 10 times as low as the other level, but even a daily intake per rat of 0.0265 gram of unsaponifiable fraction gave an average healing of zero. The irradiated unsaponifiable fraction was assayed for vitamin D at different levels, one 10 times as low as the other level, but even a daily intake per rat of 0.00158 gram gave an average healing of zero.

The spectrophotometric curves (Fig. 1) show no evidence of the presence of vitamin D or pro-vitamin D. The maximum shown in the region of 2600 A, accentuated in the curve representing the irradiated material, may presumably be due to phytosterol.

The colorimetric tests gave negative evidence for presence of vitamin D or pro-vitamin D in the unsaponifiable fraction.

SUMMARY

The vitamin D and pro-vitamin D content of avocado oil has been investigated by various methods and found to be negligible.

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LOSSES OF ASCORBIC ACID AND FOUR B VITAMINS IN VEGETABLES AS A RESULT OF DEHYDRATION, STORAGE, AND COOKING[1]

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The investigation here reported was begun early in 1942 when it became evident that light-weight foods of high nutritive value would be needed for the armed forces abroad and for economy of tin, transportation, and labor at home.

The five vegetables chosen for study were carrots, spinach, broccoli, green peas, and snap beans. Except for carrots these vegetables are not easily kept in their natural state but must be frozen or canned, and their suitability for dehydration had not then been established. The problems examined were (1) the effect of various pretreatments on the quality of the dehydrated product and (2) the effect of pretreatment, reconstitution, and storage on the quality and vitamin content of the product.

In all cases the vegetables were obtained locally. Recent cytological investigations by Mann and Weier (1944) show that few procedures for procurement are devoid of uncertainty, particularly in the case of carrots. Roots pulled at the same time from a given planting of supposedly pure seed stock show great variation with respect to such factors as starch and carotene, and presumably therefore with respect to many other factors. This makes it difficult at times to determine whether a given treatment is suitable for the vegetable, regardless of source, for maximum quality retention in the stored product. The vegetables were sampled, pretreated, dehydrated, cooked in the laboratory, immediately examined for palatability and vitamins, and portions were stored at three different temperatures for three or four months, after which they were re-examined. Usually two or more lots of each vegetable were sampled, dehydrated and assayed.

The details of blanching, dehydration, and reconstitution of these foods are presented elsewhere.[2] This report covers the vitamin studies with a brief discussion of the method of assay; a summary of source of vegetables and methods of dehydration is given (Table 1).

The vitamins studied were ascorbic acid, thiamin, riboflavin, nicotinic acid, pantothenic acid, and carotene.

Ascorbic acid was determined by titration with 2.6 dichlorophenolindophenol of a blended, one-per cent, metaphosphoric acid extract, cleared and treated with H_2S to include dehydroascorbic acid.[3]

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[1] This study was supported in part by a grant from the Bureau of Home Economics, U. S. Department of Agriculture, Washington, D. C.

[2] The dehydration was carried out by Hazel E. Friar and the cooking and palatability experiments by Judith Stillman, Betty M. Watts, and Agnes F. Morgan. A report of the latter work appeared in the Journal of Home Economics 36, 28-34 (1944).

[3] Assistance in the chemical tests for ascorbic acid, thiamin, and riboflavin was rendered by J. M. Sugihara, F. C. Fritz, and V. Frasinetti; and in the microbiological tests, by Marion B. Smith and Jean M. Peat.

TABLE 1

Source and Preparation of Vegetables

Vegetable	Variety	Source	Pretreatment	Dehydration
Carrots	Oxheart Imperator	San Joaquin Co., grown on sandy soil.	1) Steam blanched 8 min. 2) Unblanched	In home evaporator with kerosene heater in 4 to 8 hr. at 160-140°F.
Spinach	Hollandia	San Joaquin Co., grown on sandy soil.	1) Steam blanched 4 min. 2) Brine dip, boiling 1% brine, 2 min. 3) Water dip, boiling distilled water 2 min. 4) Unblanched	In home evaporator in 5 to 10 hr. at 180-126°F.
Broccoli	Sprouting Italian	Alameda Co.	1) Steam blanched 5 min. 2) Brine dip, 5 min. in 1% boiling brine, reused 3) Water dip, boiling distilled water 5 min. reused 4) Unblanched	In laboratory air-blast dehydrator in 6 to 10 hr. at 155-140°F.
Peas	a) Laxton Progress b) Laxton Progress	Pismo Beach Santa Clara Co.	1) Steam blanched 5 min. 2) Unblanched	a) Home blast dehydrator in 19 hr. at 135-100° F. b) Laboratory blast dehydrator in 9 to 11 hr. at 140-120°F.
Snap beans	a) Kentucky Wonder b) Kentucky Wonder	Coachella Valley Coachella Valley	1) Steam blanched 10 min. 2) Boiling water 10 min. 3) In 2 qt. Presto pressure cooker 5 min. 4) Unblanched	Laboratory blast dehydrator in 8 hr. at 150-140°F.

Thiamin was determined by oxidation of the extracted thiamin to thiochrome and measurement of its fluorescence according to the technique described by Conner and Straub (1941).

Riboflavin was determined by the fluorimetric technique described by Conner and Straub (1941), as modified by Mackinney and Sugihara (1942). The microbiological method developed by Snell and Strong (1939) was also used.

Nicotinic acid was determined by the microbiological method of Snell and Wright (1941) and *pantothenic acid* by that of Strong, Feeney, and Earle (1941).

Carotene was estimated by chemical and biological means and the results are reported separately.

Cooking: Unless otherwise indicated, the cooking liquid was not included in the vitamin determinations of cooked vegetables. The dehydrated vegetables were usually soaked in four or five times their volume of warm water and cooked five to 20 minutes in the same water after two to five hours of soaking.

Storage: All dehydrated samples were stored in tightly closed, screw-top glass jars in air at 0, 20, and 30°C.(32, 68, and 86°F.).

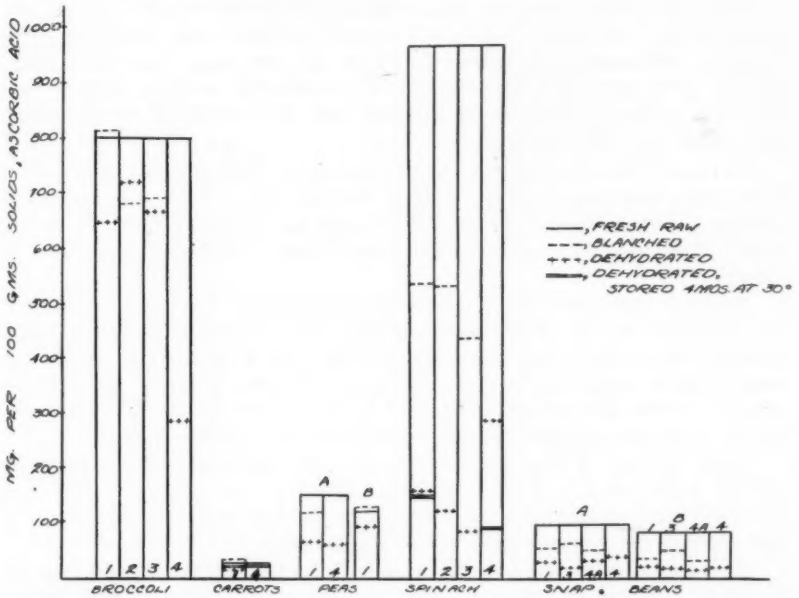

FIG. 1. Retention of ascorbic acid by vegetables during blanching, dehydration, and, in some cases, storage.

1. Steam blanched, four to 10 minutes
2. Blanched in boiling one-per cent salt solution two to five minutes
3. Blanched in boiling distilled water two to five minutes
4. Unblanched

4A. Blanched in Presto pressure cooker five minutes.

Moisture determinations were made by drying samples to constant weight in covered aluminum dishes in the vacuum oven at 70 to 80°C. (158 to 176°F.).

ASCORBIC ACID

The loss of ascorbic acid in cooking, blanching, dehydration, and storage varied with the vegetable (Fig. 1). An occasional occurrence was an apparent increase in the vitamin content of the steam-blanched vegetable over the raw fresh. No dehydroascorbic acid could be detected in any of these vegetables. Whenever the blanched or cooked vegetable yielded

a higher ascorbic acid value than the raw, the higher value was accepted as the basis on which to calculate losses. It has recently been shown by Cruickshank (1943) that the biological and chemical assays are in agreement for dehydrated cabbage (presumably freshly prepared). The presence of foreign reducing substances which yield erroneously high values with the dye titration is well known. The product stored at one temperature may sometimes show a peculiar discrepancy compared with those stored at higher or at lower temperatures. It is well, therefore, to consider such values as "apparent."

Broccoli: This vegetable was difficult to sample because of its unhomogeneous character, but the blanched samples lost relatively little ascorbic acid during dehydration, 11 to 20 per cent. The unblanched sample of broccoli lost 64 per cent of its ascorbic acid in dehydration. Thus the properly dehydrated broccoli may be considered an unusually rich source of this vitamin.

Carrots: This vegetable is poorly endowed with ascorbic acid in the fresh state and lost about one-half in dehydration. There was no loss in storage in the blanched product and even an apparent increase in one case. The unblanched dehydrated carrots also underwent little loss in storage.

Green Peas: Two lots of peas were dehydrated: a mature lot, A, and an immature lot, B, of the same variety. The mature peas had a larger ascorbic acid value than the immature but the final product of the immature peas retained 50 per cent more vitamin, possibly because the mature lot was dehydrated in a small home dehydrator and the immature lot in a commercial-type tunnel dehydrator in a shorter time.

Snap Beans: Two lots—mature, A, and immature, B—were dehydrated unblanched and after steam, water-dip, and pressure-cook types of blanching. In all cases the dehydrated product retained only 20 to 30 per cent of the ascorbic acid originally present.

Spinach: About one-half of the ascorbic acid of the spinach was lost in blanching and 30 per cent more in dehydration. The blanched dehydrated spinach in all cases had lost 84 to 91 per cent of the vitamin and the unblanched 70 per cent, but in storage the ascorbic acid of the latter continued to decrease whereas the blanched product suffered less change.

THIAMIN

The problems of extraction of the full thiamin content of vegetables preparatory to its chemical determination are specific to each vegetable. The usual 0.04 N H^2SO_4 treatment is sufficient in the case of simple plant tissues, such as leaves and nonstarchy roots, but is inadequate for samples containing much starch or protein. Enzymatic treatment is necessary in the latter case. In several vegetables, better extraction of thiamin was possible in the steamed or cooked product than in the raw.

Broccoli: The thiamin content (Fig. 2) of the steam-blanched sample was greater than that of the raw and was therefore accepted as the maximum. The steam-blanched dehydrated product contained 78 per cent of the original thiamin but the brine-blanched and water-blanched products had only 45 and 42 per cent. In storage the unblanched dehydrated broc-

coli lost 40 per cent and the steam-blanched 43 per cent at 30°C.(86°F.). The steam blanching was most advantageous for thiamin retention.

Spinach: This vegetable, which was the best source of thiamin examined, also lost less when steam blanched before dehydration than when water or brine blanched. Again about one-third disappeared in the best product and very little more was lost in storage.

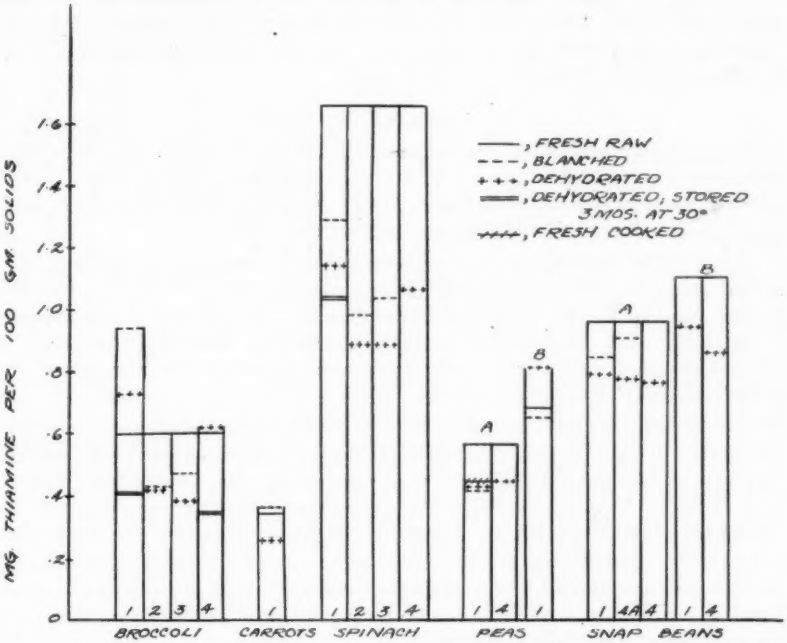

FIG. 2. Retention of thiamin by vegetables during blanching, dehydration, and, in some cases, cooking and storage. (Columns numbered as in Fig. 1.)

Peas: The peas were somewhat lower in thiamin than those examined by Fincke, Little, Redelings, and Perkins (1943) by biological assay, who found 2.0 to 7.1 micrograms per gram in several varieties of peas and no loss owing to freezing or cooking. Barnes, Tressler, and Fenton (1943) reported about four micrograms per gram in fresh and three in frozen and stored peas with 80 to 84 per cent retention in the fresh cooked samples, all determined by the thiochrome method. Moyer and Tressler (1942) also found that the biological method yielded larger values, 4.22 and 3.06 micrograms per gram as compared with 2.6 micrograms per gram, by the thiochrome method.

These peas, Laxton's Progress variety, had one to two micrograms per gram of thiamin in the fresh state and retained 70 to 75 per cent in dehydration in the home dehydrator and apparently all in the commercial-type dehydration. In the latter case an apparent increase occurred, possibly owing to better extraction of thiamin from the dehydrated than from the raw samples.

Green Snap Beans: Two lots of beans of the Kentucky Wonder variety—mature, A, and immature, B—were examined. The blanched dehydrated beans contained more than 80 per cent of the original thiamin and the unblanched, 75 per cent. Farrell and Fellers (1942) found by biological assay 68 per cent retention of thiamin in blanched dehydrated beans of the Bountiful variety. Moyer and Tressler (1942) compared the thiochrome, biological, and fermentation methods of thiamin assay in fresh green beans and found 0.76 microgram per gram by bioassay, 0.64 by thiochrome, and 0.63 by fermentation, as compared with about 1.2 by thiochrome in the beans reported on here.

RIBOFLAVIN

Some difficulties were encountered in the fluorimetric procedure because the recommended adsorbent was not available at first (Fig. 3). As noted by Mackinney and Sugihara (1942), however, fairly consistent readings could be obtained directly on the extracts.

Peas: The loss of riboflavin was small in cooking, blanching, and dehydrating, but there appeared to be a chance of about 50-per cent loss in the reconstitution of the dehydrated peas. No riboflavin disappeared in storage. Both microbiological and fluorimetric methods were used on all pea samples with satisfactory agreement in most cases, although the microbiological values tended to be larger.

Snap Beans: Only microbiological technique was used on the beans. The immature lot, B, contained more than twice as much as the mature, A, and retained about 75 per cent of it throughout the processing. Farrell and Fellers (1942) reported 143 per cent of the original riboflavin in blanched dehydrated beans, a result obtained by rat-growth technique.

Carrots: Carrots, also examined only by microbiological means, although poorly endowed with riboflavin, retained it well during dehydration but lost about half in the cooking of the dehydrated vegetable as well as in the cooking of the fresh vegetable.

Spinach: Dehydration of spinach after steam blanching and without blanching produced a total loss of 24 to 40 per cent of the riboflavin present in the fresh vegetable, but larger losses were encountered in the reconstitution of the dehydrated product. No changes occurred in storage. The agreement between results obtained by fluorimetric and microbiological methods was excellent.

Broccoli: The fresh cooked, steam-, water-, or brine-blanched vegetable retained about two-thirds of its riboflavin with little further loss in dehydration, in reconstitution, or in storage. The values obtained by the fluorimetric and microbiological methods were in less consistent agreement in the case of this vegetable than in that of spinach, probably because of the unavoidable errors of sampling.

Thus, agreement between the microbiological and fluorimetric assays appeared to vary with the concentration of riboflavin in the vegetable. Excellent agreement was obtained with spinach which supplied 2.0 to 2.4 mg. of riboflavin per 100 gm. dried sample; fair agreement was obtained with broccoli which supplied 1.0 to 1.5; and the least satisfactory agree-

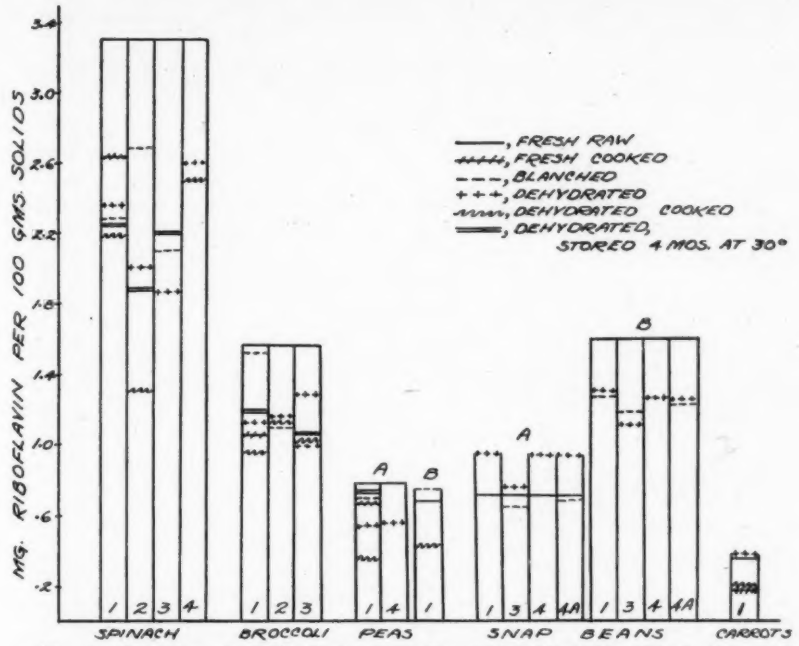

FIG. 3. Retention of riboflavin by vegetables during blanching, dehydration, and, in some cases, cooking and storage. (Columns numbered as in Fig. 1.)

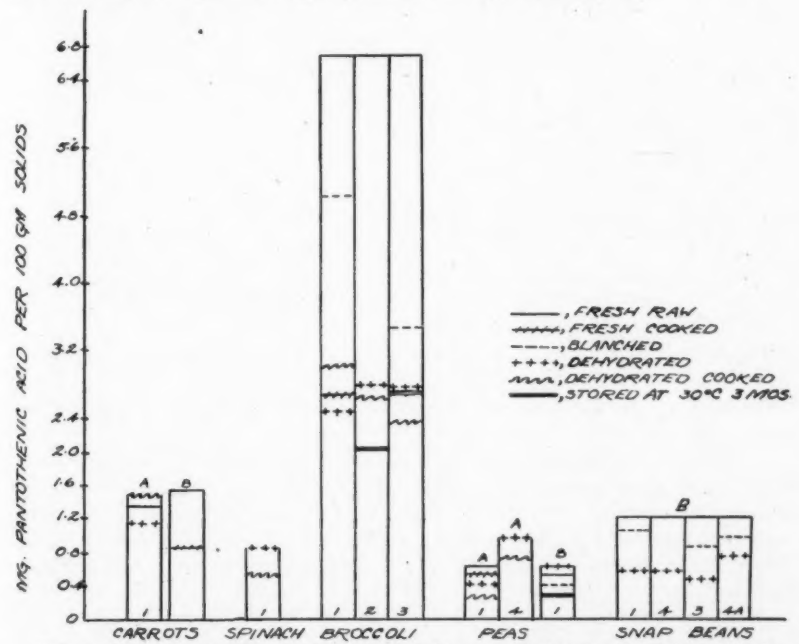

FIG. 4. Pantothenic acid content of vegetables as affected by blanching, dehydration, and, in some cases, cooking and storage. (Columns numbered as in Fig. 1.)

ment was obtained in the case of peas which contained 0.3 to 0.7 mg. per 100 gm. dried sample.

PANTOTHENIC ACID

Only a few samples were examined for this vitamin, all by the microbiological technique (Fig. 4).

Broccoli: Broccoli was found to be outstanding in its content of this vitamin, 40 to 60 per cent of which was lost in cooking, blanching, and dehydrating. There remained, however, usually 2 to 4 mg. per 100 gm.

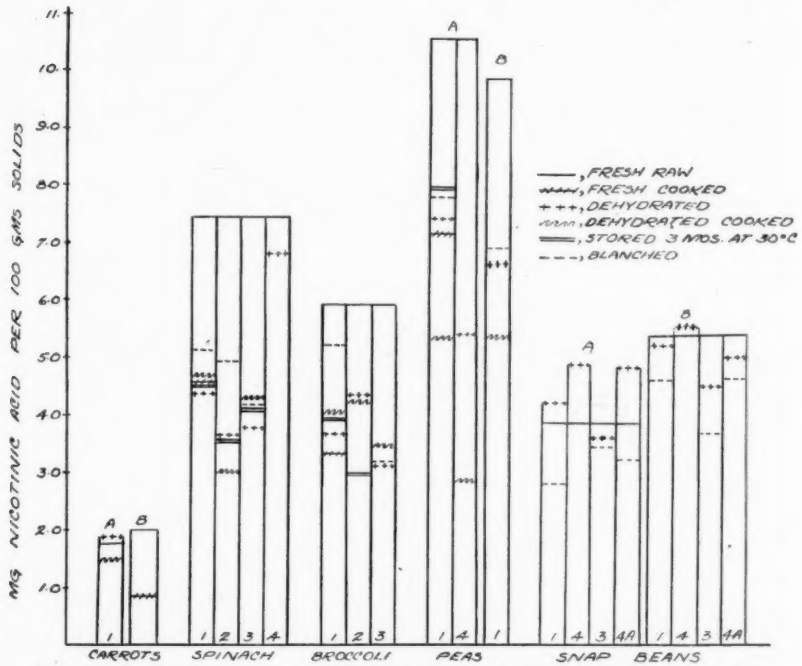

FIG. 5. Nicotinic acid content of vegetables as affected by blanching, dehydration, and in some cases, cooking and storage. (Columns numbered as in Fig. 1.)

in the dehydrated product, little of which was removed when the vegetable was cooked in moderate amounts of water. The reconstituted dehydrated broccoli was equivalent to the cooked fresh product. There was little loss in storage.

Carrots, snap beans, spinach, and *peas* appeared to contain pantothenic acid in measurable amounts. Peas lost very little during dehydration or cooking but about one-fourth in storage. The snap beans, however, appeared to lose about one-half the vitamin in dehydration, except in the sample, 4A, which was sliced lengthwise and steam-pressure blanched.

Jukes (1941), by a chick test, found similar values for pantothenic acid: in broccoli 4.6 mg. per 100 gm. dry matter, carrots 1.3, and spinach

1.0, as compared with our broccoli which contained 6.6, carrots 1.3, and spinach 0.8. Cheldelin and Williams (1942), by the microbiological method, found in carrots 2.1, green peas 1.5, spinach 1.7 mg. of pantothenic acid per 100 gm. dry matter.

NICOTINIC ACID

The *peas, spinach,* and *broccoli* were found to be well endowed with nicotinic acid as judged by the results of the microbiological assays (Fig. 5). Little difficulty was encountered in making the extractions and most of the determinations were satisfactorily consistent.

TABLE 2

Vitamin Content of Fresh, Cooked, and Dehydrated Vegetables

Vegetable	Moisture	Ascorbic acid	Thiamin	Riboflavin	Pantothenic acid	Nicotinic acid
	pct.	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Carrots						
Fresh raw	87.2	3	0.04	0.04	0.16	0.24
Fresh cooked or steam blanched	80.2	4	0.04	0.04	0.17	0.18
Dehydrated	3.8	15	0.23	0.36	1.10	1.80
Reconstituted	81.2	....	....	0.04	0.28	0.28
Spinach						
Fresh raw	91.5	81	0.14	0.28	....	0.63
Fresh cooked or steam blanched	91.3	57	0.14	0.24	....	0.40
Dehydrated	2.6	155	1.10	2.30	0.84	4.30
Reconstituted	80.7	....	....	0.42	0.08	0.86
Broccoli						
Fresh raw	87.2	102	0.08	0.20	0.85	0.75
Fresh cooked or steam blanched	89.1	101	0.12	0.11	0.29	0.35
Dehydrated	3.3	623	0.70	1.02	2.35	3.65
Reconstituted	80.7	....	....	0.19	0.58	0.80
Peas						
Fresh raw	79.0	28	0.13	0.15	0.12	2.15
Fresh cooked or steam blanched	80.6	28	0.12	0.13	0.10	1.45
Dehydrated	6.3	71	0.59	0.46	0.51	7.00
Reconstituted	70.9	....	....	0.11	0.15	1.51
Snap beans						
Fresh raw	87.6	12	0.13	0.13	0.11	0.54
Fresh cooked or steam blanched	89.8	8	0.17	0.13	0.12	0.53
Dehydrated	4.7	23	0.82	1.07	0.54	4.51

In all five vegetables there were measurable losses, probably owing to leaching of the nicotinic acid from the fresh cooked samples. Steam or water blanching produced a similar loss, 30 to 50 per cent, but very little further loss in dehydration, reconstitution, or storage.

Tepley, Strong, and Elvehjem (1942) reported values obtained by a similar method as follows: green beans 6.09 mg. of nicotinic acid per 100 gm. dry matter, carrots 14.0, spinach 7.65, as compared with our green beans 3.85 and 5.40, carrots 1.75 and 2.00, and spinach 7.41. Russell, Taylor, and Beuk (1943) found 1.4 mg. per 100 gm. in raw peas, 0.35 in raw green beans, 0.20 to 0.35 in raw carrots, 0.8 and 1.0 in raw broccoli, 0.7 in raw spinach on the fresh basis. All of these values check satisfactorily with those obtained in our laboratory.

A summary is given (Table 2) of the vitamin analyses for fresh raw (frozen), fresh cooked or steam blanched, freshly dehydrated, and reconstituted or cooked dehydrated forms of the five vegetables. Only the results of chemical and microbiological methods are included in this table since sufficient data by biological assay were not available for comparison.

SUMMARY

Ascorbic Acid: Snap beans and spinach lost 70 to 90 per cent of their ascorbic acid in dehydration regardless of blanching, peas and carrots lost about 50 per cent, and blanched broccoli lost 20 per cent or less and unblanched 64 per cent. The fresh and dehydrated cooked vegetables were not examined for this vitamin. Unblanched vegetables, spinach and carrots, tended to lose the vitamin in dehydration and storage more readily than the blanched. Blanching with steam usually caused less loss than brine or water blanching.

Thiamin: Steam-blanched dehydrated vegetables lost less thiamin than the water-blanched or unblanched dehydrated samples. These five vegetables retained 65 to 80 per cent of the original thiamin throughout the dehydration process. Broccoli lost about 40 per cent additional after storage for three months at 30°C., but spinach had negligible losses of thiamin in similar storage.

Riboflavin: Spinach and broccoli were the best sources of riboflavin among the five vegetables studied and they lost 20 to 35 per cent of it in being cooked fresh or in dehydration and reconstitution. Snap beans, also a good source, retained 75 per cent or more in the dehydrated product. Carrots and peas contained less riboflavin; they lost a small amount in steam blanching and very little in dehydration but nearly one-half of that left during reconstitution. There was no significant loss in storage in the spinach, broccoli, and peas.

Pantothenic Acid: Pantothenic acid was retained by dehydrated reconstituted broccoli to the extent of 2 to 3 mg. per 100 gm., or 40 per cent of the fresh value. Carrots, snap beans, peas, and spinach had about the same amount of the vitamin in the fresh state, 0.12 mg. per 100 gm., and retained most of it during dehydration, except for snap beans which lost about one-half. Blanched dehydrated broccoli lost little in storage for three months at 30°C.(86°F.), but one sample of blanched dehydrated peas lost 25 per cent in similar storage.

Nicotinic Acid: Nicotinic acid was lost about equally, 20 to 50 per cent, by ordinary cooking and by dehydration and reconstitution. Practically none disappeared during storage for three months at 30°C. in blanched dehydrated spinach, broccoli, and peas.

CONCLUSIONS

Riboflavin, pantothenic acid, and nicotinic acid are likely to be retained during the process of blanching and dehydrating of vegetables about as well as during ordinary cooking of the fresh vegetables. Losses of ascorbic acid and thiamin are usually somewhat greater in unblanched dehydrated vegetables than in blanched, particularly in storage, but this is not true of riboflavin, pantothenic acid, and nicotinic acid.

Leaching during the blanching process, particularly if water dipping is used, may cause greater loss of these four B vitamins than does enzyme catalyzed oxidation in the unblanched samples.

Only ascorbic acid and thiamin, and to a lesser degree pantothenic acid, were found to decrease in the stored dehydrated vegetables.

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EFFECTS OF AGING, FREEZING RATE, AND STORAGE PERIOD ON PALATABILITY OF BROILERS [1]

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Frozen liver as well as breast and thigh meat of broilers has been observed to be less palatable than that of fresh chicken held 24 hours at 1.7°C.(35°F.)—Hanson, Stewart, and Lowe (1942) and Stewart, Lowe, and Morr (1941). The palatability changes involve two factors, flavor and juiciness of the meat. The change in flavor occurs in dressed poultry which has been chilled and frozen within two to five hours after slaughter as well as in poultry which has been chilled promptly, then eviscerated and frozen within 18 hours—Lowe (1939).

The lack of juiciness is a "dryness" in texture of frozen and stored poultry meat. Although noticeable in the thigh muscles, it is more pronounced in the breast muscles. The "dryness" is more noticeable in some birds than in others. In extreme cases the meat is exceedingly tender but the texture feels powdery, somewhat like fine sawdust. The microscopic appearance of cooked muscle fibers of birds showing this "dryness" is normal, but the appearance of the elastic fibers is different from that observed in raw or cooked, fresh or aged, unfrozen poultry muscles. The elastic fibers seem to have partially disintegrated and are found in short, narrow, needle-like rods instead of the normal, long, slender, apparently never-ending fibers. Lowe (1943) has suggested that the cause of the partial disintegration of the elastic fibers in stored frozen poultry is enzymatic. If true, since the partial disintegration of the elastic fibers has been observed only in frozen poultry, the fraction of the proteolytic enzyme acting on elastin must be fairly active at low temperatures.

The work of Koonz and Ramsbottom (1939) and of DuBois, Tressler, and Fenton (1942) indicates that only an extremely rapid freezing rate preserves the integrity of the muscle fibers. McClurg (1941) found beef to be more palatable and ice crystals smaller at lower storage temperatures. He recommends temperatures below —20.5°C.(—5°F.) for storage of frozen beef. In work on beef, Moran and Hale (1932) found that during storage there is a gradual increase in ice-crystal size, which is more rapid as the storage temperature is elevated. This change in ice-crystal size may be partially responsible for the change in texture of frozen poultry muscle.

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The work of Sair and Cook (1938) indicates that aging before freezing markedly affects the amount of "drip" obtained from poultry during defrosting.

In view of the foregoing facts, it seemed desirable to investigate the combination of aging before freezing, the rate of freezing, and the length of the storage period on the texture and flavor of poultry meat.

EXPERIMENTAL PROCEDURE

Preparation and Freezing: Thirty-six New Hampshire broilers (2.5 to 3.5 lb.) were selected from a large commercial flock near Cambridge, Massachusetts. They were killed, semiscalded, plucked, then eviscerated and quartered at once. They were then placed in latex (Cry-o-vac) bags. The bags were evacuated and the open end twisted tight and heat sealed. Half of the birds were placed in a cooler at 1.7°C.(35°F.) for 18 hours before freezing.

The birds were then handled by the following three methods:

1. Twelve broilers were frozen in a dry ice-alcohol mixture at —67.8°C. (—90°F.).
   a) Six were held less than two hours before freezing.
   b) Six were held 18 hours before freezing.
2. Twelve broilers were frozen in an air blast at —45.6°C.(—50°F.).
   a) Six were held less than two hours before freezing.
   b) Six were held 18 hours before freezing.
3. Twelve broilers were frozen in uncirculated air at —20.5°C.(—5°F.).
   a) Six were held less than two hours before freezing.
   b) Six were held 18 hours before freezing.

As soon as frozen these lots were packed in Meese shipping containers with dry ice and shipped by express to Ames. The frozen broilers were removed from the containers and immediately placed at —23.3°C.(—10°F.) for the duration of the storage period. There was no evidence of thawing during transit; large pieces of dry ice were still in the containers when they were opened.

Palatability Technics: At the end of 9, 23, 37, 51, 65, and 79 days after slaughter, one bird from each of the six treatments was removed and thawed (in package) in an electric refrigerator at 4.4 to 7.2°C.(40 to 45°F.) for 48 hours. The amount of drip was measured and the appearance and odor of various parts of the carcass were scored. The quarters of each chicken were cooked in a gas oven at 160°C.(320°F.) until the temperature at the center of the thigh reached 85°C.(185°F.) (cooked in an open pan, skin side down for the first 30 minutes, then turned to finish). The livers were cooked in 75 milliliters of water in the top of a double boiler for 15 minutes.

Pieces from the thigh, breast, and liver were given to each of the judges (the first three authors) to score for odor, flavor, tenderness, and juiciness. Each judge always received his samples from the same anatomical part of the various muscles being scored. A 10-point scoring system was used, 10 being perfect and zero extremely unpalatable.

Fresh controls (broilers killed and plucked, then eviscerated at once, and held at 1.7 to 4.4°C. for 24 hours) were used in each comparison. The

controls were coded, as were all other birds under test, so that bias on the part of the judges was greatly minimized.

Histological Sections: For the microscopic study of the tissues four sections were prepared from each bird. They consisted of raw and cooked tissues from the pectoralis major of the breast and the gluteus primus of the thigh. The paraffin sections were differentially stained to show the elastic and collagenous fibers and the muscle fibers as dark blue-green, red or magenta, and yellow-orange, respectively. The stains used were French's modification of Weigert's elastic tissue stain, van Giessen's mixture of acid fuchsin and picric acid, and Harris's alum hemotoxylin.

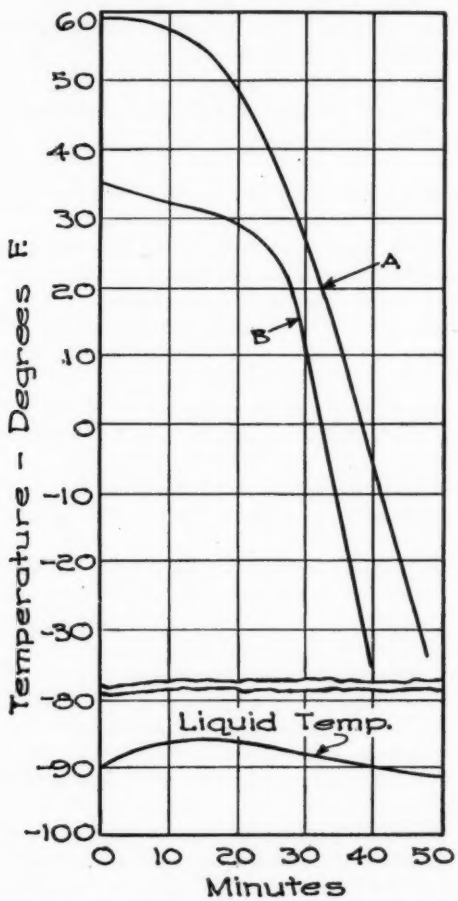

FIG. 1. Freezing rates of quartered broilers in solid carbon dioxide-alcohol mixture, —67.8°C.(—90°F.).

Curve A—placed in freezing mixture two hours after slaughter;

Curve B—placed in freezing mixture 18 hours after slaughter.

RESULTS

Freezing Rates: The rates of freezing for the three temperatures used are shown graphically (Figs. 1, 2, and 3). The range of freezing time was great, from about 10 minutes for the solid carbon dioxide-alcohol mixture (the time during the actual freezing represented by the plateau on the curves) to approximately five hours for the birds frozen in uncirculated air.

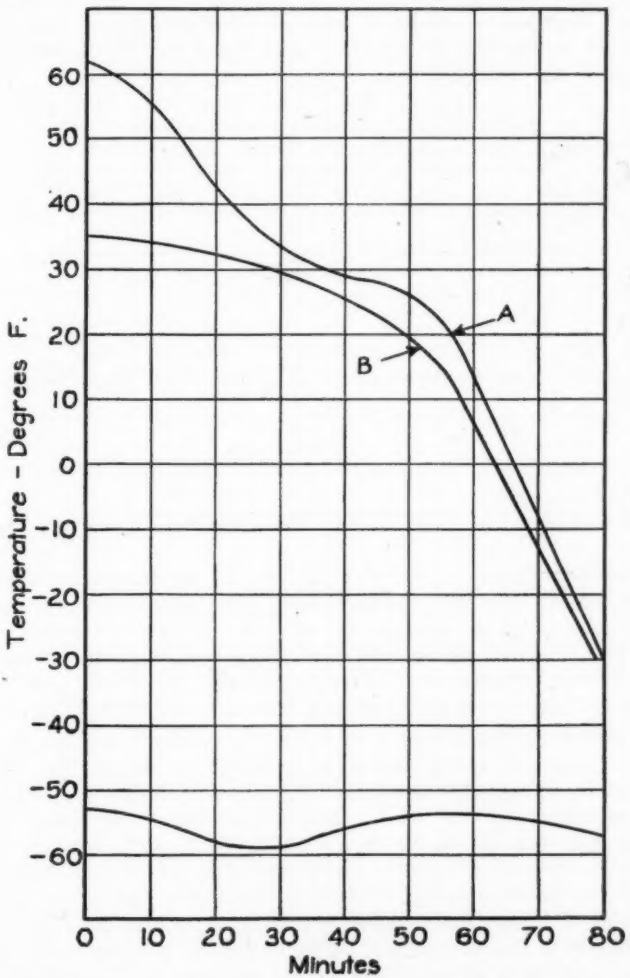

FIG. 2. Freezing rates of quartered broilers in air blast (3,300 ft. per min.), —45.5°C.(—50°F.).

Curve A—placed in air blast two hours after slaughter;

Curve B—placed in air blast 18 hours after slaughter.

The temperature drop (Curve A, Figs. 1, 2, and 3) was slower for the broilers frozen within two hours after slaughter than for the birds held 18 hours before freezing. This may be related to the greater post-mortem changes in the birds held the longer time before freezing. Hanson *et al.* (1942) found the rate of heat penetration in broilers was slower in birds held a short time before cooking than in those held for a longer period.

Appearance and Drip: The appearance of all birds was excellent when thawed except that the bones and the meat adjacent to the bones were commonly dark when compared with the fresh controls. There was no evidence of desiccation at any time and there was no measurable drip from any of the thawed birds.

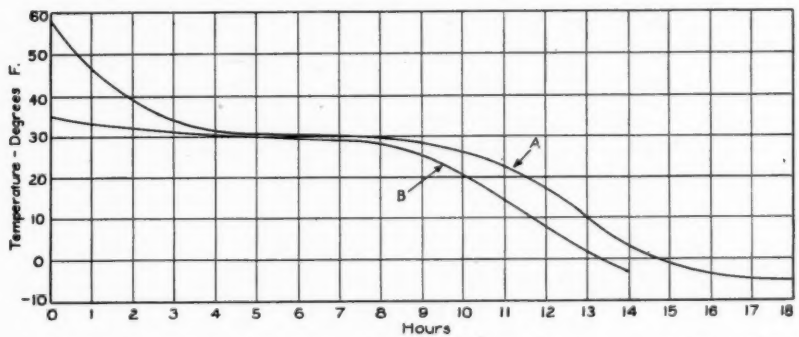

FIG. 3. Freezing rates of quartered broilers in uncirculated air, —20.5°C.(—5°F.).
Curve A—placed in freezer two hours after slaughter;
Curve B—placed in freezer 18 hours after slaughter.

Palatability Scores: The effect of the six treatments on palatability was determined from the judges' scores. The significance of the results was determined by the analysis of variance; a typical analysis is shown (Table 1). The effect of the treatment (fresh control *versus* treated, aging time, freezing temperature, and storage time) on breast juiciness and flavor, thigh juiciness and flavor, and liver flavor is shown (Table 2).

The aroma and tenderness scores are not given in the tables. In general the aroma scores show the same trend as the flavor scores. The treatments caused no significant differences in tenderness of the muscles, except that the thigh muscles of the fresh controls were rated less tender than the corresponding muscles in the frozen birds. In an earlier study, reported by Lowe (1939), all muscles of birds held overnight before freezing were more tender (as determined by both scores and shearing strength) than the muscles of birds frozen within two hours after killing. The differences in the results of the two studies, owing to time of holding before freezing, may be explained in methods of handling and in age of birds used. In the earlier study roasters instead of broilers were used, the birds were not all eviscerated, and no birds were quartered before freezing. The extra handling in quartering the broilers may have hastened resolution of rigor in the birds frozen within two hours after killing. In addition, longer holding

before freezing may have had more effect in tenderizing muscles of roasters than of broilers.

The judges consistently preferred the fresh controls to the frozen birds for all palatability factors (Table 2) with the exception of tenderness of thigh muscles previously noted.

The aging time before freezing, in general, had far less effect upon the palatability scores than the length of the storage period after freezing. The greatest difference for aging before freezing was shown in thigh scores for juiciness (aging time, Table 2).

TABLE 1

Analysis of Variance. Effect of Aging, Freezing Rate, and Storage Time on Juiciness Scores of Chicken-Breast Muscle

Source of variation	Degrees of freedom		Sum of squares	Mean square
	Individual	Total		
Total	....	41	62.7	....
1. Storage period	....	5	23.8	4.76[1]
2. Treatment	....	6	16.3	2.72
a) Fresh controls *vs.* frozen	1	....	13.0	12.98[1]
b) Aging time	1	....	1.1	1.14
c) Freezing rate	2	....	1.3	0.64
d) Interaction (aging time, freezing rate, storage period)	2	....	0.9	0.46
3. Interaction	....	30	22.6	0.75
a) Length storage × aging	5	....	7.3	1.46
b) Length storage × freezing rate	10	....	5.0	0.50
c) Residual	15	....	10.3	0.69

[1] Highly significant.

The three different freezing rates used did not produce any detectable differences in any of the palatability measures used.

In the early frozen-storage periods the differences in palatability scores between the fresh controls and frozen broilers were not significant. However, with longer storage the palatability scores of the frozen broilers decreased, the differences becoming highly significant for all palatability factors after 51 days of storage.

The flavor of the frozen liver rapidly deteriorated with storage, the differences between the scores for fresh and frozen liver becoming highly significant after 23 days of storage.

Histological Sections: The microscopic study showed that the rate of freezing and time of aging before freezing affected the histological appearance of the muscle fibers. All birds frozen at —67.8°C. within two hours after killing had vacuoles within the fibers of both breast and thigh muscles. Vacuoles were also present in the breast tissues of all birds frozen at —45.5°C. within two hours after killing, but they were absent in half the thigh muscles of this lot.

Some of the vacuoles were lenticular in shape, the longer diameter paralleling the fiber longitudinally, so that the spaces often resembled

TABLE 2

Effect of Aging, Freezing Temperature, and Storage Time on Average Palatability Scores of Broilers [1]

Comparison	Breast				Thigh				Liver	
	Juiciness		Flavor		Juiciness		Flavor		Flavor	
	Av.	Diff.	Av.	Diff.	Av.	Diff.	Av.	Diff.	Av.	Diff.
Treatment										
Fresh control	7.2	....	9.3	....	8.2	....	9.0	....	8.8	....
Remainder	5.6	1.6[2]	7.8	1.5[2]	7.4	0.8[2]	7.2	1.8[2]	5.5	3.3[2]
Aging time (hr.)										
2	5.4	....	7.6	....	7.1	....	7.3	....	5.6	....
18	5.7	+0.3	8.1	+0.5[3]	7.6	0.5[3]	7.2	0.1	5.5	0.1
Freezing temperature (°C.)										
—20.5	5.8	....	8.0	....	7.6	....	7.3	....	5.6	....
—45.6	5.4	0.4	8.0	0.0	7.2	0.4[3]	7.1	0.2	5.4	0.2
—67.8	5.6	0.2	7.6	0.4	7.4	0.2	7.1	0.2	5.6	0.0
Storage period (days)										
Fresh control	7.2	....	9.3	....	8.2	....	9.0	....	8.8	....
9	7.1	0.1	9.0	0.3	8.5	+0.3	9.4	+0.4	7.9	0.9
23	6.6	0.6	8.5	0.8[3]	8.1	0.1	8.3	0.7	6.7	2.1[2]
37	5.4	1.8[2]	8.5	0.8[3]	7.4	0.8	7.5	1.5[3]	6.1	2.7[2]
51	5.3	1.9[2]	7.6	1.7[2]	7.1	1.1[2]	6.8	2.2[2]	5.9	2.9[2]
65	5.2	2.0[2]	7.5	1.8[2]	6.7	1.5[2]	6.6	2.4[2]	4.0	4.8[2]
79	5.1	2.1[2]	7.4	1.9[2]	7.0	1.2[2]	6.3	2.7[2]	5.3	3.5[2]

[1] Range of scores is from 0 to 10, with 10 high. [2] Highly significant. [3] Significant.

beads strung in a row (Figs. 4 and 5). Other vacuoles were cylindrical in shape, so that the fiber appeared as if it had been split longitudinally, i.e., a slender column of ice had formed within the fiber (Fig. 5). The lenticular-shaped vacuoles were far more numerous than the cylindrical ones. In some birds the vacuoles were very numerous in both the raw and cooked sections from the breast and thigh muscles. In other birds, as has been indicated above, the vacuoles were more numerous in the breast than in

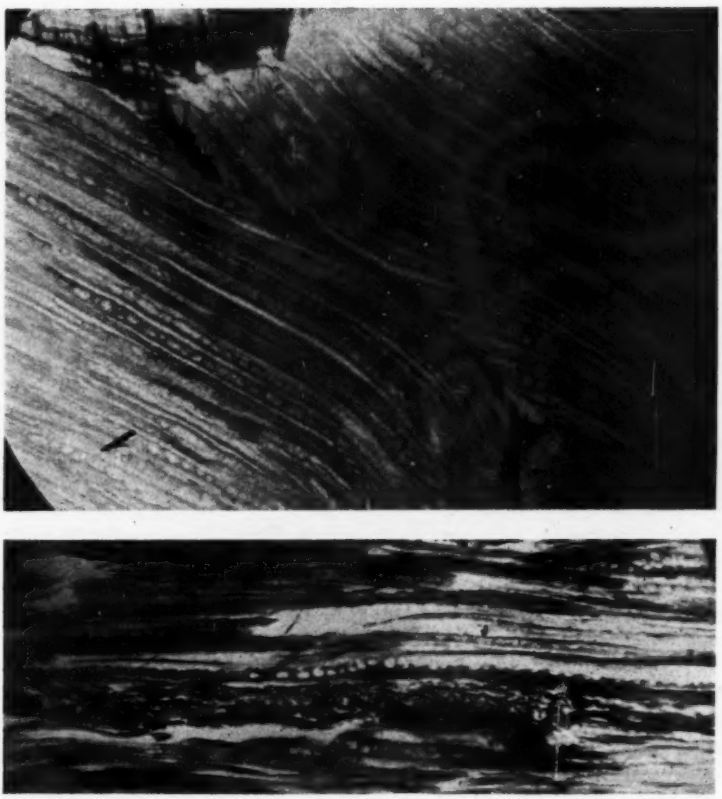

FIG. 4. Photomicrographs showing numerous vacuoles within fibers of longitudinal sections: upper, of cooked pectoralis major muscle from a broiler frozen at —67.8°C. (—90°F.) within two hours after slaughter; lower, of a raw gluteus primus muscle frozen under the same conditions. The separation of the elastic fibers (black) into criss cross patterns has been observed only in poultry that has been frozen and stored (×80).

the thigh muscle fibers. In some birds the vacuoles occurred in occasional fibers or in certain parts of a fiber, whereas most of the fibers presented the normal appearance with no empty spaces except the breaks characteristic of aging.

There were practically no vacuoles in the muscle fibers of the broilers held 18 hours before freezing. It took careful and intensive inspection of the sections to find the few vacuoles in an occasional fiber (some were found in three birds frozen at —67.8°C. and in one bird frozen at —45.5°C.). In fact, if all birds had been held 18 hours before freezing, the few vacuoles found in the fibers would have been considered an abnormality of the fiber, faulty technic, or would have been overlooked.

No vacuoles were found in the fibers of birds frozen at —20.5°C. regardless of the time of aging before freezing.

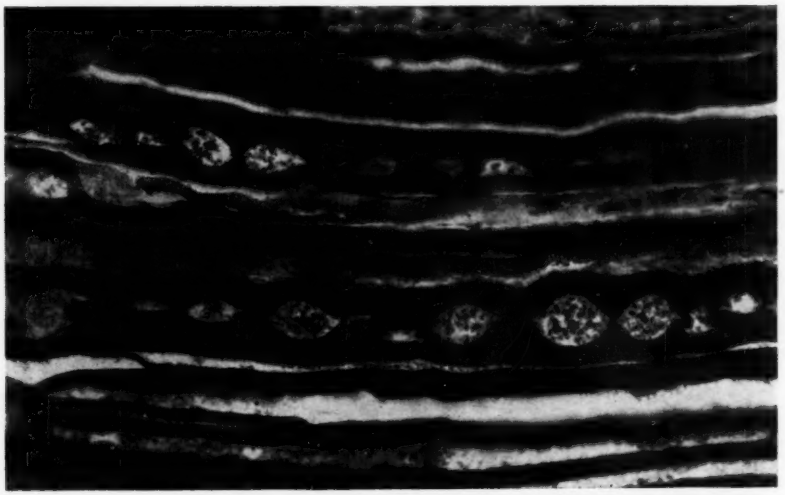

FIG. 5. Photomicrograph of longitudinal section of pectoralis major muscle of broiler frozen at —67.8°C.(—90°F.) within two hours after killing, showing the lenticular and cylindrical vacuoles (×410).

It seems probable that the vacuoles occurred where ice crystals had previously been formed. Muscle fibers shrink in diameter during cooking and also in the dehydrating agents used in preparing the sections. In shrinking the fiber would tend to separate leaving empty spaces at the site formerly occupied by ice crystals. If the formation of the vacuoles can be taken as an indication of intra-fibrillar freezing, then in addition to the rate of freezing or freezing temperature the extent of the post-mortem changes also affects the formation of intra-fibrillar ice crystals.

Shrewsbury *et al.* (1942) observed both intra- and extra-fibrillar freezing in pork muscle. The intra-fibrillar freezing was indicated by vacuoles in the fibers, previously occupied by ice formations. The appearance of the sections indicated the ice columns were arranged lengthwise of the fibers. Sometimes more than one column was found in the same fiber. These investigators state they found considerable variation in the frozen tissues. This was attributed to differences in individual animals and the length of frozen storage (all cuts were frozen at —26°F.), but extent of the post-mortem changes at time of freezing was not considered.

Both passive and active rigor nodes as reported by Paul, Lowe, and McClurg (1944) were observed in the histological sections of the broilers. The waves and kinks of passive rigor (Fig. 6) were nearly always found near connective tissue, either collagenous or elastic or both, as if the contractures might have been caused by shrinking of the connective tissues. The waves and kinks were found in tissues of all broilers regardless of time held before freezing or the temperature of freezing. The active rigor nodes (Fig. 7) were found more frequently in broilers frozen within two hours after the birds were killed.

Hanson, Stewart, and Lowe (1942) have presented photomicrographs showing the disintegration of the striated to a granular-like structure in muscle fibers of broilers in which rigor has been resolved. In the present study, the thin spots and breaks in muscle fibers, which are characteristic of the passing of rigor, were far more numerous in birds held 18 hours than in those held two hours before freezing. The breaks (Fig. 7) were more numerous in the breast than in the thigh muscle fibers. In the uncooked tissue the cross striations have disappeared in the thin spots and in the breaks of the fibers, leaving (as far as the staining technics show) an apparently empty space. Cooking further alters the histological appearance of the fibers, in that a granular-like tissue replaces the apparently empty spaces of breaks of the uncooked tissues. Cooking also accentuates the falling apart of the elastic fibers into the short, needle-like rods shown in the illustrations.

SUMMARY

Thirty-six broilers were divided into two groups; one group was held 18 hours before freezing whereas the other was frozen within two hours after killing. Each group was divided into three lots; one lot was frozen at —67.8°C.(—90°F.), another at —45.6°C.(—50°F.), and the third at —20.5°C.(—5°F.). At the end of 9, 23, 37, 51, 65, and 79 days after slaughter one bird from each of the six lots was removed from storage, thawed, cooked, and scored for palatability. A fresh unfrozen broiler was always used for a control.

The freezing time varied from about 10 minutes for birds frozen at —67.8°C. to approximately five hours for those frozen at —20.5°C.

The fresh controls were consistently preferred to the frozen broilers for all palatability factors, i.e., aroma, flavor, juiciness, and tenderness, with exception of tenderness of the thigh muscles.

The aging before freezing had no effect on the palatability scores.

The three freezing rates used did not produce detectable differences in the palatability scores.

In the early frozen-storage periods the differences in palatability scores between the fresh controls and the frozen broilers were not significant. With longer storage the scores for the frozen broilers decreased, the differences between the fresh and frozen birds becoming highly significant for all factors after 51 days of storage.

The microscopic study showed that both the rate of freezing and time of aging before freezing affected the histological appearance of the muscle fibers. All birds frozen at —67.8°C. within two hours after slaughter had

FIG. 6. Passive rigor, waves, in fibers of cooked thigh muscle of broiler frozen at —45.6°C.(—50°F.) within two hours after killing. The collagenous tissue on both sides is partially hydrolyzed to gelatin by cooking (×200).

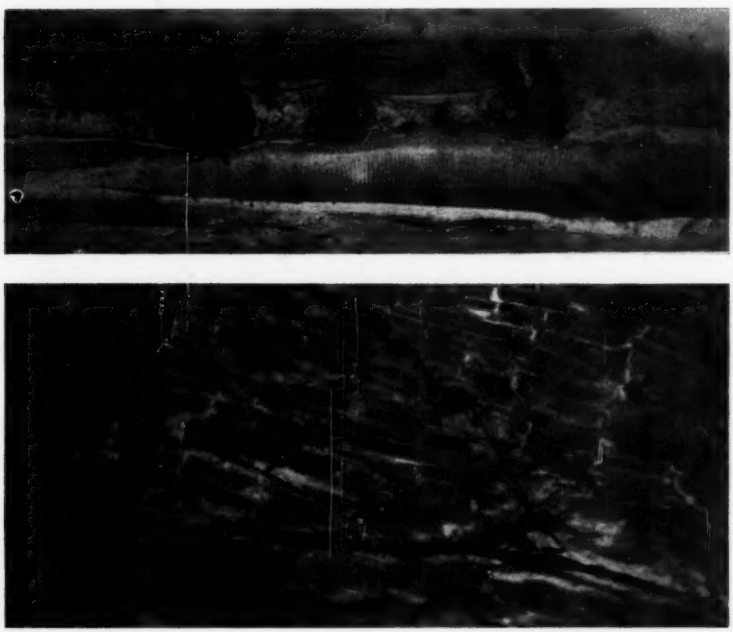

FIG. 7. Upper: Active rigor nodes characterized by alternate areas of contracture and rarefaction; loss of cross striations occurs first in rarefied areas; from the thigh muscle (cooked) of broiler frozen within two hours after killing (×300).

Lower: Breaks in muscle fibers and elastic fibers (black); from cooked breast muscle of broiler held 18 hours before freezing (×80).

vacuoles within the fibers of breast and thigh muscles. The vacuoles were very numerous in both raw and cooked sections from some broilers, less so in other broilers. Sometimes the number of vacuoles was greater in the breast than in the thigh muscles. These vacuoles were considered an indication of intra-fibrillar freezing, ice crystals having formerly occupied the site of the vacuoles. Intra-fibrillar freezing also occurred in all breast muscles and half of the thigh muscles of broilers frozen within two hours after killing at —45.5°C. No intra-fibrillar freezing occurred in broilers frozen within two hours after killing at —20.5°C. In general, intra-fibrillar freezing did not occur in any broilers held 18 hours before freezing, regardless of the freezing temperature used.

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USE OF PEANUT FLOUR IN BAKING

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The natural habitat of the peanut is hard to determine, though authorities generally agree that it is a native of Brazil whence it was introduced into Europe and later into Africa. It was brought to the United States and disseminated in this country during colonial times, probably having been included in the ship stores of slave traders.

The peanut is an annual herbaceous plant belonging to the Phili-pionacae, a suborder of the natural order Leguminosae and botanically known as *Arachis hypogaea.* Two types of peanuts are grown in the United States—the Virginia type, which includes such well-known varieties as Virginia Bunch, Virginia Runner, and North Carolina, and the Spanish type, which includes the true Spanish, Georgia, Tennessee Red, and Valencia varieties. The peanut is not a nut but a legume like the pea and bean and in its chemical composition, especially its high protein and fat contents, it resembles the soybean more than other legumes.

A number of investigators have conducted feeding experiments which indicate that the protein of the peanut is of high nutritive value. Johns and Finks (1920) found that a diet containing wheat flour as the only source of protein and water-soluble vitamins, together with adequate inorganic salt and butterfat, produced only one-third to two-thirds normal growth in rats. Bread made with mixtures of 25 parts peanut flour and 75 parts wheat flour furnished adequate protein and water-soluble vitamins for normal growth. Eddy and Eckman (1923) conducted experiments using rations containing only 10 per cent of protein, six to seven per cent of this protein being contributed by wheat flour and the rest by peanut flour or meat residue (supplemented by three per cent butterfat and three per cent mineral salts). The peanut flour proved slightly superior to the meat for growth and markedly superior for reproduction. Daniels and Loughlin (1918) concluded from a feeding experiment with rats that peanuts are a complete food except for vitamin A and minerals. In an investigation of wheat-flour supplements, Jones and Divine (1942) conducted an experiment on rats in which flours were incorporated in the diet so as to supply 9.1 per cent protein. To those fed patent wheat flour only the average gain in weight was 19 grams in 42 days, while the rats which were fed 90 per cent patent wheat flour and 10 per cent peanut flour gained 44 grams, or more than twice as much in the same period of time. Zucker and Zucker (1943) conducted feeding experiments using peanut flour in the diet of rats and recommended the use of peanut flour as a supplement for wheat flour in human diets.

Burr, Brown, Kass, and Lundberg (1940) and Quackenbush, Kummerow, and Steenbock (1942) have shown that linoleic acid is essential in

TABLE 1

Chemical Composition of Wheat Flours, Wheat-Flour Supplements, and Some Ingredients Used in Bread

Kind of flour or ingredient	Moisture	Protein	Ash	Fat	Carbohydrates		Calcium	Phosphorus	Iron	Thiamin[1]	Riboflavin	Niacin
					Crude fiber	Other carbohydrates						
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*µg./ 100 gm.*	*µg./ 100 gm.*	*µg./ 100 gm.*
Enriched patent flour	13.0	11.2	0.45	1.0	0.20	74.15	0.017	0.100	.00136	366	45	1,358
Optional enriched patent flour	13.0	11.2	0.45	1.0	0.20	74.15	0.108	0.100	.00136	366	269	1,358
Patent flour	13.0	11.2	0.45	1.0	0.20	74.15	0.017	0.100	.0008	70	45	1,100
Clear flour	13.0	13.6	0.73	2.0	0.33	70.45	0.023	0.171	.0019	265	75	2,400
Whole-wheat flour	10.5	12.1	1.9	2.0	2.4	71.10	0.047	0.421	.0037	485	115	6,500
Wheat germ	9.3	28.9	4.6	11.0	2.0	44.20	0.067	1.152	.0086	2,400	650	6,300
Peanut flour	6.0	56.8	4.0	8.6	4.0	21.30	0.127	0.800	.0035	750	350	25,000
Soya flour	7.0	49.2	5.4	6.5	2.7	29.30	0.309	0.727	.0080	750	400	5,500
Cottonseed flour	7.0	52.0	6.0	6.5	3.0	25.50	0.218	.....	.....	1,040	1,020	8,500
Dry skim milk	4.2	35.6	8.0	1.5	....	50.70	1.272	0.923	.0035	357	2,060	1,830
Yeast, compressed	70.0	17.3	3.4	1.0	....	8.30	0.030	0.712	.0031	795	1,800	20,000

[1] The abbreviation, µg., in this and other tables refers to micrograms.

nutrition and that it must be included in the diet. The linoleic acid content of peanut oil is from 22 to 25 per cent, which is much higher than that of animal fats.

The carbohydrate content of the peanut is low compared with that of most other seeds, the range as reported in the literature being six to 24 per cent. Neale (1926), who made a special study of the uses of peanuts as a diabetic food, found a carbohydrate content of 22.95 per cent. Neale's findings indicate that only 6.7 per cent of the total carbohydrates was utilizable by man. Smith and Wilder (1926) conducted feeding experiments in which peanuts were fed to a diabetic, and found that they were a useful food in the diet of such patients. These authors support the validity of Neale's conclusion and for clinical purposes accept her figure of 6.7 per cent for the absorbable carbohydrates of peanut flour.

Sherwood and Halverson (1939); Higgins, Holley, Pickett, and Wheeler (1941a,b); Pickett (1941); Booher (1941); Booher, Hartzler, and Hewston (1942); McVicar and Berryman (1942); Payne (1942) and others have studied the vitamin content of peanuts and find them to be an excellent source of thiamin, riboflavin, and niacin.

TABLE 2

Amount of Food Constituents Furnished by Adding Various Quantities of Dry Skim Milk to One Pound of Bread

Amount dry skim milk added	Protein	Calcium	Phosphorus	Iron	Thiamin	Riboflavin	Niacin
	gm.	*gm.*	*gm.*	*gm.*	*µg.*	*µg.*	*µg.*
6.3 grams or 2%.....	2.2	.08	.06	.0002	23	130	115
12.6 grams or 4%.....	4.5	.16	.12	.0004	45	260	230
18.9 grams or 6%.....	6.7	.24	.17	.0006	68	389	346
25.2 grams or 8%.....	9.0	.32	.23	.0008	90	519	461

Peanut flour, like peanut meal, is the product left after the oil has been extracted. Peanut flour differs from peanut meal in that it is prepared under sanitary conditions and from skinned peanuts. It is an excellent product from a nutritional standpoint. It has about ten times the mineral content of patent wheat flour, five times as much protein, and is much richer in vitamins. Peanut flour is an excellent supplement for wheat flour as it supplies most of the constituents that are lacking in wheat flour. The composition of wheat flours, peanut flour and other wheat-flour supplements, and some of the ingredients of bread are shown (Table 1). This table was compiled from data received through correspondence with manufacturers and from data found in the literature. Data from more than two hundred publications were used in preparing this table. A rather wide range was found for most of the constituents listed. The data were studied and values which appeared to represent an average were selected.

Clear wheat flour may be used to better advantage than patent wheat flour in making bread containing peanut flour. Clear flour contains more gluten than patent flour and is higher in vitamins and minerals.

Dry skim milk added to bread enhances the nutritional value of the bread. Milk is especially high in riboflavin as compared with most other food products. Six per cent of dry skim milk is regarded as the preferable

amount to be used in bread, but there is not enough dry skim milk available at the present time to put this amount in all bread. Data on the amounts of protein, calcium, phosphorus, iron, thiamin, riboflavin, and niacin supplied to one pound of bread by dry skim milk when added in quantities of two, four, six, or eight per cent are shown (Table 2).

ANALYSIS OF PEANUT FLOUR

Three samples of commercially prepared peanut flour were analyzed by the author. The average results, in per cent, were as follows: moisture, 7.5; protein, 58.0; fat, 9.6; ash, 3.7; crude fiber, 2.1; total sugar as sucrose, 7.3; other carbohydrates (by difference), 11.8; calcium, 0.074; phosphorus, 0.556; and iron, 0.0033.

ANALYSIS OF COMPONENT PARTS OF THE PEANUT

Five samples of Spanish and four samples of Virginia peanuts were sorted; the trash and light-weight hulls were removed; the peanuts were then separated by hand into shell, skin, germ, and cotyledon. The average percentage of each component part was calculated and chemical analyses were made on each (Table 3).

Twenty-nine per cent of the Virginia type and only 20 per cent of the Spanish type were found to be composed of shell. The proportion of skins was about the same for the two types, both being 1.8 per cent. The germ comprised 3.4 and 1.7 per cent of the Spanish and Virginia types, respectively. Seventy-five per cent of the Spanish peanut and 67 per cent of the Virginia peanut were found to be composed of cotyledon.

The fat content of the cotyledon of the Spanish type (49.5 per cent) was higher than that of the Virginia type (46.3 per cent). Most investigators have found somewhat greater differences in the fat content of these two types of peanuts.

The protein contents, in per cent, of separated parts of the Spanish and Virginia types, respectively, were as follows: shell, 4.8 and 5.6; skin, 11.0 and 13.4; germ, 27.8 and 26.5; and cotyledon, 31.9 and 30.6. The ash contents of the germ were 3.2 (Spanish) and 2.9 (Virginia) per cent, which were considerably higher than that found in the other component parts of these peanuts. The crude-fiber contents of the cotyledon and of the germ for both types of peanuts were about the same, the range being from 1.6 to 1.9 per cent. The skin of the Spanish peanut was somewhat more fibrous than that of the Virginia peanut, the crude-fiber contents being 21.4 and 17.3 per cent, respectively. On the other hand, the shell of the Virginia peanut contained more crude fiber (74.3 per cent) than that of the Spanish peanut (67.4 per cent). The germ and cotyledon contained only a trace of reducing sugar in both types, whereas the skins contained about one per cent. The total sugar (after inversion) in the germ averaged 7.9 per cent for the two types and 6.2 per cent in the cotyledon for the two types. The total sugar in the skins and shells averaged less than two per cent for the two types.

Peanuts are high in calcium and iron compared with most other food products. Since calcium and iron are two of the minerals most likely to be lacking in the diet, their presence in relatively large amounts adds to their value as a food. The calcium contents, in per cent, of the various

TABLE 3

Analysis of Component Parts of Spanish and Virginia Types of Peanuts

Type and component part	Proportions of component parts	Moisture	Protein (N x 6.25)	Ash	Fat	Carbohydrates		Sugar		Calcium	Phosphorus	Iron
						Crude fiber	Other carbohydrates	Before inversion	After inversion			
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Spanish												
Shell	20.30	9.3	4.8	2.2	1.3	67.4	0.76	1.8	13.2	129	52	8.3
Skin	1.75	10.2	11.0	2.1	1.9	21.4	1.2	1.2	52.2	380	57	7.5
Germ	3.35	4.3	27.8	3.2	43.0	1.8	Trace	7.9	12.0	64	625	3.4
Cotyledon	74.60	3.5	31.9	2.2	49.5	1.6	Trace	5.0	6.3	46	393	2.0
Virginia												
Shell	29.30	4.4	5.6	1.9	2.8	74.3	0.9	1.6	9.4	127	39	2.8
Skin	1.84	7.4	13.4	2.1	0.5	17.3	1.0	1.0	48.3	385	61	4.8
Germ	1.70	3.5	26.5	2.9	43.0	1.6	Trace	7.9	14.5	80	568	3.6
Cotyledon	67.10	3.7	30.6	2.2	46.3	1.9	Trace	7.4	7.9	44	411	2.1

component parts of the two types of peanuts averaged as follows: shell, 0.128; skin, 0.383; germ, 0.072; and cotyledon, 0.045. The iron contents of the cotyledon and germ averaged 0.0021 and 0.0035 per cent, respectively. The skins and shells of the Spanish type contained much more iron than did the same parts of the Virginia type. Much more phosphorus was found in the cotyledon and germ than in the skins and shells.

EXPERIMENTS ON USE OF PEANUT FLOUR IN BAKING BREAD

Peanut flour in proportions up to 40 per cent can be used with white wheat flour for making bread. The method used in making bread was essentially that employed by L. H. Bailey, formerly of this Bureau, for wheat-flour supplements.

The formula and method used in baking were as follows:

Sponge

Flour	200 gm.
Water	130 ml.
Yeast	8 gm.
Dry malt extract	1 gm.
Ferment sponge 180 minutes	

Dough

Flour	47.0 gm.
Peanut flour	64.0 gm.
Salt	6.3 gm.
Sugar	9.5 gm.
Shortening	6.3 gm.
Water	35.0 ml.
Dry skim milk	18.9 gm.
Ferment dough 20 minutes	
Proof for 65-70 minutes	

The same ingredients were used when the bread was made by the straight dough procedure. By this procedure all the ingredients were added at the same time. The doughs were fermented 140 minutes and proofed for 65 to 70 minutes. The bread made by the straight dough procedure was slightly better than that made from the sponge dough. Satisfactory bread can be made by either procedure.

Photographs of bread made with all clear wheat flour and with mixtures of clear flour and peanut flour (Fig. 1) show that there is a slight decrease in the whiteness of the crumb as the amount of peanut flour is increased. Peanut flour does not contain gluten; therefore, with a decrease in amount of wheat flour and a corresponding increase in peanut flour there is a decrease in the volume of the loaf.

Peanut flour may also be used with whole-wheat flour. Photographs of bread made with all whole-wheat flour, and with a mixture of peanut and whole-wheat flours (Fig. 2) show a slight decrease in volume as a result of adding peanut flour. The grain and texture of the loaf containing peanut flour were as good as in the loaf made of whole-wheat flour only.

EXPERIMENTS ON USE OF GROUND PEANUTS AND PEANUT FLOUR IN QUICK BREAD AND CAKE

The peanuts were roasted and the skins removed. Then they were ground in a household food grinder. The ground product as well as a

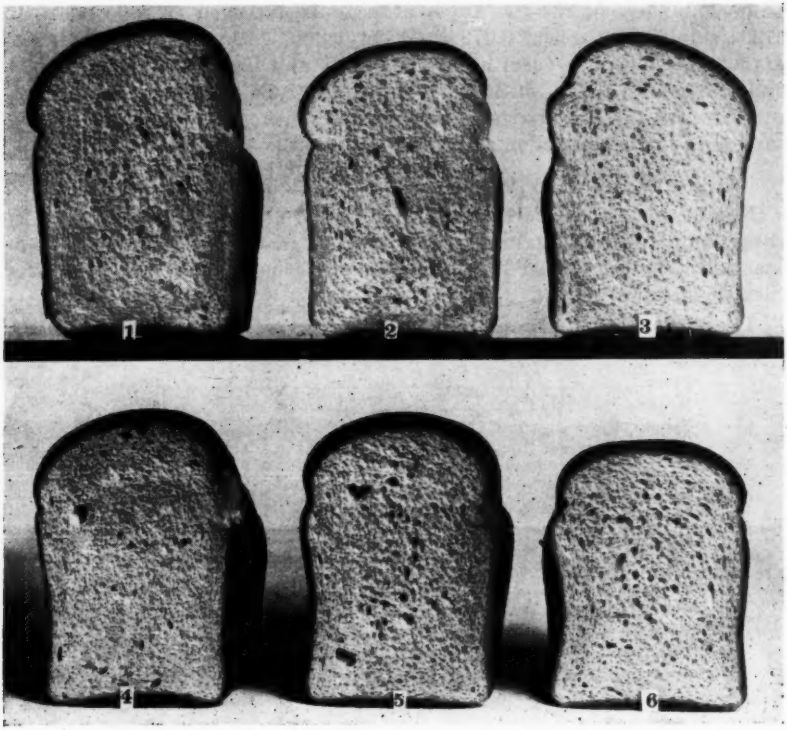

FIG. 1. Bread made with all clear wheat flour and with mixtures of peanut and clear wheat flours.

100% clear flour
95% clear, 5% peanut
90% clear, 10% peanut
85% clear, 15% peanut
80% clear, 20% peanut
75% clear, 25% peanut

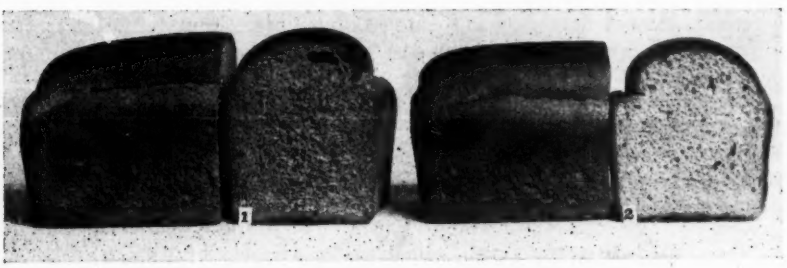

FIG. 2. Bread made with all whole-wheat flour and with a mixture of peanut and whole-wheat flours.

100% whole-wheat flour
85% whole-wheat flour, 15% peanut flour

commercial peanut flour were incorporated in such products as biscuits, muffins, griddle cakes, waffles, and sugar cookies. Satisfactory products were made when proportions of 50 per cent wheat flour and either 50 per cent peanut flour or 50 per cent ground peanuts were used; photographs of cookies, biscuits, and cakes are shown (Figs. 3, 4, and 5), respectively.

Freshly sifted peanut flour is lighter in weight than soft wheat flour. One and one-third cups of peanut flour weigh approximately the same as one cup of wheat flour. Recipes for biscuits and other baking powder-leavened products can be converted over to recipes containing peanut flour by allowing for this difference in weight of the two products.[1]

CALCULATED COMPOSITION OF BREADS CONTAINING FLOUR SUPPLEMENTS

The composition of breads made from patent, clear, or whole-wheat flours and mixtures of these individual flours with peanut flour are presented (Table 4). The data for composition (Table 1) were used in calculating the amount of the food constituents in 100 grams of bread. The milk and yeast used in making the bread contribute to the vitamin, mineral, and protein contents of the bread as well as to the flour.

The calculations show that the bread made from all clear flour contains about three times as much thiamin, about twice as much niacin or iron, and slightly more protein, calcium, and phosphorus than the bread made from all patent flour.

By comparing the bread made of 80 per cent clear flour and 20 per cent peanut flour with that made of the same proportions of patent flour (80 per cent) and peanut flour (20 per cent), the calculations also show that the bread containing clear flour is a decided improvement in nutritive value over that containing patent flour.

The bread made from a mixture of 95 per cent clear wheat flour and five per cent peanut flour contains three per cent more protein than the bread made from enriched patent wheat flour; otherwise this bread has about the same nutritional value as that made from enriched patent flour. Breads containing 10, 15, 20, or 25 per cent peanut flour are of increasingly higher nutritional value.

The data (Table 4) also show that bread made with whole-wheat flour or a mixture of whole-wheat flour (85 per cent) and peanut flour (15 per cent) is a very highly nutritious food.

Wheat germ, soya flour, and cottonseed flour, like peanut flour, may be used as wheat-flour supplements. These flours contain larger quantities of minerals, vitamins, and protein as well as protein of higher nutritional value than that of wheat flour. The calculated food constituents in 100-

[1] The National Peanut Council, Inc., of Atlanta 3, Georgia, has issued two booklets in which are listed recipes using peanuts and peanut products. The titles are "Peanuts—Their Food Value and Interesting Recipes" and "Peanuts—Some Ideas for Use in Quantity Cookery." These booklets may be secured by writing directly to the Council. The Georgia Agricultural Experiment Station—Ascham (1936)—has also published suitable recipes for use of peanut flour in baked products.

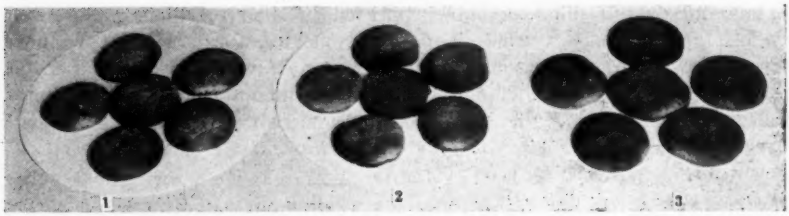

FIG. 3. Sugar cookies made with all patent wheat flour and with a mixture of peanut flour or ground peanuts with patent wheat flour.

100% patent flour	50% patent flour 50% peanut flour	50% patent flour 50% ground peanuts

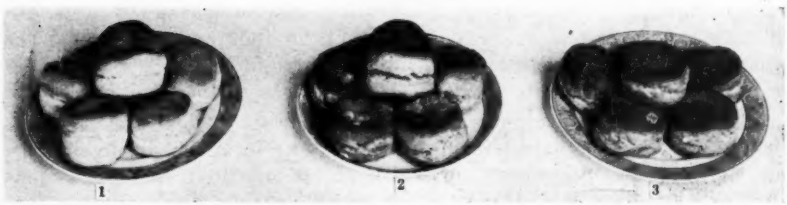

FIG. 4. Biscuits made with all patent wheat flour and with a mixture of peanut flour or ground peanuts with patent wheat flour.

100% patent flour	50% patent flour 50% peanut flour	50% patent flour 50% ground peanuts

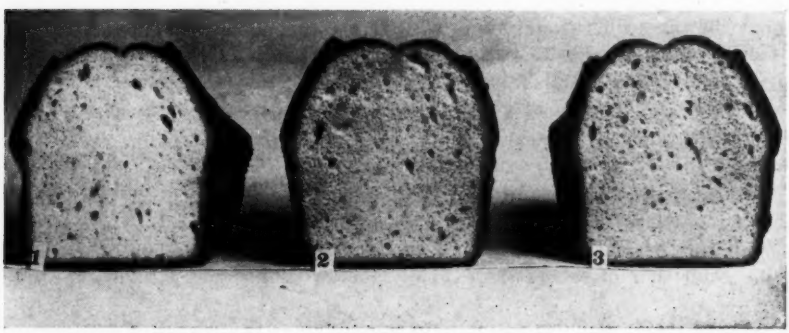

FIG. 5. Cake made with all patent wheat flour and with a mixture of peanut flour or ground peanuts with patent wheat flour.

100% patent flour	80% patent flour 20% peanut flour	80% patent flour 20% ground peanuts

TABLE 4

Food Constituents in 100-Gram Portions of Bread Made From Different Types of Flour and From Same Flours Supplemented With Peanut Flour

Types of flour and supplements	Protein	Ash (NaCl free)	Fat	Carbohydrates		Calcium	Phosphorus	Iron	Thiamin	Riboflavin	Niacin
				Crude fiber	Other carbohydrates						
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*μg.*	*μg.*	*μg.*
Enriched patent flour, 100%	9.6	0.7	0.8	0.1	52.7	.065	.120	.0011	240	149	1,363
Optional enriched patent flour, 100%	9.6	0.8	0.8	0.1	52.7	.129	.120	.0011	240	304	1,363
Patent flour, 100%	9.6	0.7	0.8	0.1	52.7	.065	.120	.0007	66	149	1,187
Patent flour, 80%; peanut flour, 20%	15.6	1.2	1.8	0.7	45.5	.081	.218	.0011	146	191	4,507
Clear flour, 100%	11.2	0.9	1.5	0.2	50.2	.070	.169	.0015	181	169	2,090
Clear flour, 95%; peanut flour, 5%	12.7	1.0	1.7	0.4	48.5	.073	.191	.0016	196	179	2,875
Clear flour, 90%; peanut flour, 10%	14.2	1.1	1.9	0.5	46.8	.077	.213	.0016	209	188	3,660
Clear flour, 85%; peanut flour, 15%	15.7	1.2	2.1	0.6	45.1	.080	.237	.0017	224	198	4,444
Clear flour, 80%; peanut flour, 20%	17.2	1.4	2.4	0.7	43.5	.084	.257	.0018	238	207	5,229
Clear flour, 75%; peanut flour, 25%	18.7	1.5	2.6	0.9	41.8	.088	.279	.0018	252	217	6,014
Whole-wheat flour, 100%	10.2	1.7	1.5	1.7	50.6	.086	.343	.0028	311	197	4,937
Whole-wheat flour, 85%; peanut flour, 15%	14.8	1.9	2.2	1.8	45.5	.095	.383	.0028	320	222	6,864

gram portions of bread as affected by replacement of 20 per cent of the wheat flour with flour supplements is shown (Table 5).

About one-third of a loaf of bread per person is consumed daily in the United States in normal times. The working week has been lengthened from 40 to 48 hours. With the increase in working hours there is an increase in the need for energy-producing foods; bread is one of the best foods of this type. Assuming that the daily consumption of bread during war time is one-half pound per person, data were calculated showing the amount of protein, calcium, phosphorus, iron, thiamin, riboflavin, and niacin that this amount of bread will yield. These data, as well as the daily requirements for these constituents as set by the National Nutrition Council are presented (Table 6). These calculations were made on the basis of the bread containing six per cent of dry skim milk; Table 2 contains data for use in adjusting the figures when quantities other than six per cent of dry skim milk are used.

The National Nutrition Council has set the daily requirement for protein as 70 grams for a man weighing 154 pounds (70 kilograms). One-half pound of bread made from patent wheat flour contains 22 grams of protein or 31 per cent of the daily requirement, whereas bread containing 5, 10, 15, 20, or 25 per cent of peanut flour will furnish 41, 46, 51, 56, or 61 per cent, respectively, of the daily requirement. The peanut flour furnishes more protein, and this protein is of much higher nutritional value than that of wheat flour. Johns and Finks (1920), Eddy and Eckman (1923), Suzuki, Matsuyama, and Hashimoto (1925), Daniels and Loughlin (1918), Jones and Divine (1942), Zucker and Zucker (1943), and others have amply demonstrated the superiority of peanut protein to that of wheat-flour protein.

SUMMARY

Peanut flour is an excellent flour from the nutritional standpoint. It contains about ten times the mineral content of patent wheat flour, five times as much protein, and is much richer in vitamins. Peanut flour added to wheat flour is an excellent supplement as it compensates for some of the nutritional value lacking in wheat flour.

Clear wheat flour may be used to better advantage than patent wheat flour in making bread containing peanut flour as it contains more gluten than patent flour and is higher in vitamins and minerals.

Analyses of three samples of commercially prepared peanut flour were made. The average results, in per cent, were as follows: moisture, 7.5; protein, 58.0; fat, 9.6; ash, 3.7; crude fiber, 2.1; total sugar as sucrose, 7.3; other carbohydrates (by difference), 11.8; calcium, 0.074; phosphorus, 0.556; and iron, 0.0033.

Spanish and Virginia peanuts were separated into their various component parts (shell, skin, germ, and cotyledon) and analyzed. The shell content of the Spanish type was lower than that of the Virginia type; the skin was the same in both; the germ and cotyledon contents of the Spanish type were greater than those of the Virginia peanut. The skins and shells were high in calcium and low in phosphorus compared with the germ and cotyledon.

TABLE 5

Food Constituents in 100-Gram Portions of Bread as Affected by Replacement of 20 Per Cent of Wheat Flour With Flour Supplements[1]

Types of flour and supplements	Protein	Ash (NaCl free)	Fat	Carbohydrates		Calcium	Phosphorus	Iron	Thiamin	Riboflavin	Niacin
				Crude fiber	Other carbohydrates						
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*μg.*	*μg.*	*μg.*
Enriched patent flour, 100%	9.6	0.7	0.8	.1	52.7	.065	.120	.0011	240	149	1,363
Clear flour, 100%	11.2	0.9	1.5	.2	50.2	.070	.169	.0015	181	169	2,090
Clear flour, 80%; peanut flour 20%	17.2	1.4	2.4	.7	43.5	.084	.257	.0018	238	207	5,229
Clear flour, 80%; wheat germ, 20%	13.4	1.4	2.7	.5	46.6	.076	.306	.0024	433	249	2,632
Clear flour, 80%; soya flour, 20%	15.1	1.4	2.0	.5	41.7	.102	.231	.0022	222	200	2,357
Clear flour, 80%; cottonseed flour, 20%	16.6	1.6	2.1	.6	44.0	.097	.....	.....	272	300	2,938

[1] Calculations based on presence of six per cent of dry skim milk in bread.

TABLE 6

Food Constituents in Half-Pound Portions of Breads Made From Wheat Flours Alone or Wheat Flours Plus Flour Supplements[1]

Types of flour and supplements	Protein		Calcium	Phosphorus	Iron	Thiamin	Riboflavin	Niacin
	Amount	Daily requirement						
	gm.	*pct.*	*gm.*	*gm.*	*gm.*	*μg.*	*μg.*	*μg.*
Enriched patent flour, 100%	21.7	31	.148	.272	.0026	545	337	3,100
Optional enriched patent flour, 100%	21.7	31	.292	.272	.0017	545	690	3,100
Patent flour, 100%	21.7	31	.148	.272	.0026	149	337	2,693
Whole-wheat flour, 100%	23.1	33	.195	.778	.0063	704	447	11,198
Clear flour, 95%; peanut flour, 5%	28.9	41	.166	.434	.0036	444	405	6,520
Clear flour, 90%; peanut flour, 10%	32.3	46	.174	.484	.0037	475	427	8,300
Clear flour, 85%; peanut flour, 15%	35.7	51	.182	.538	.0039	508	449	10,075
Clear flour, 80%; peanut flour, 20%	39.1	56	.190	.583	.0040	540	471	11,860
Clear flour, 75%; peanut flour, 25%	42.5	61	.199	.632	.0041	573	492	13,639
Whole-wheat flour, 85%; peanut flour, 15%	33.7	48	.214	.869	.0063	725	503	15,569
Clear flour, 80%; wheat germ, 20%	30.3	43	.171	.694	.0056	981	564	5,969
Clear flour, 80%; soya flour, 20%	34.2	49	.231	.524	.0045	503	453	5,346
Clear flour, 80%; cottonseed flour, 20%	37.6	54	.219	.....	.....	617	681	6,662
Daily requirement (National Nutrition Council)	70	....	0.8	1.5	0.012	1,500	2,700	20,000

[1] Calculations based on presence of six per cent of dry skim milk in bread.

Very satisfactory bread, biscuits, muffins, cookies, and cake were made from mixtures containing peanut flour and wheat flour.

A table giving the composition of wheat flours, wheat-flour supplements, and some of the ingredients of bread (dry skim milk and compressed yeast) was prepared from data found in the literature and obtained through correspondence with manufacturers. From these data other tables were prepared showing the calculated amounts of protein, calcium, phosphorus, iron, thiamin, riboflavin, and niacin in 100-gram and half-pound portions of bread made from various kinds of flour and various combinations of wheat flour and flour supplements.

A half-pound portion of bread made from patent wheat flour contains 31 per cent of the daily protein requirement for a man weighing 154 pounds (70) kilograms), whereas bread containing 5, 10, 15, 20, or 25 per cent peanut flour will furnish 41, 46, 51, 56, or 61 per cent, respectively, of the daily requirement. Bread containing peanut flour will serve as a very satisfactory substitute for meat.

Peanuts are high in calcium, iron, and vitamins compared with most other food products.

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ASCORBIC ACID CONTENT OF FRUITS OF PUERTO RICO WITH DATA ON MISCELLANEOUS PRODUCTS

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Determinations of plasma ascorbic acid values for groups of individuals of widely different economic levels indicated that for the majority of the people in Puerto Rico the dietary intake of vitamin C is less than that considered essential for best health. The present study was undertaken to obtain information on the vitamin C content of fruits and vegetables grown on the Island, with the aim of determining the most reliable and richest sources that might be used for a supply throughout the year.

Many fruits grow in abundance in Puerto Rico and some of them are in season at all times. It seemed that, if information were available concerning their ascorbic acid content, it might serve as an inducement for more extended and consistent use of them.

EXPERIMENTAL PROCEDURE

Method of Assay: Vitamin C concentration was determined by the extensively used 2,6-dichloro-phenolindophenol titration technique described by Bessey and King (1933). The visual method of titration was used for all except the few highly colored extracts since the Evelyn photoelectric colorimeter at the school was not accessible for routine work. The dye solution was measured from a rapid delivery semimicro 10-milliliter burette graduated in 0.05 milliliter, and the titration was carried out so that the end-point was reached in 10 seconds or less. A dye solution was never used longer than five days but it was generally made fresh. It was standardized each day of use by titration against aliquots of a standard solution of pure ascorbic acid, prepared in the same medium as that used for extracts of the foods. The standard solution of ascorbic acid was made at frequent and regular intervals.

Determinations on highly colored extracts were made with the Evelyn photoelectric colorimeter or the Pfaltz and Bauer fluorophotometer, according to the method described by Mindlin and Butler (1938) and Evelyn, Malloy, and Rosen (1938). Correction for turbidity was made following the plan of Bessey (1938) and Butler and Cushman (1940). The K value for the Pfaltz and Bauer fluorophotometer was determined by measurements with various dilutions of a standard solution of ascorbic acid, and the K value given for the Evelyn was checked in the same way.

Preparation of Extracts: A Waring blendor was used in the preparation of the extracts as suggested by Morell (1941), Davis (1942), and

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Loeffler and Ponting (1942). The extractant was a metaphosphoric acid solution, as originally described by Musulin and King (1936). In carrying out an analysis the food product was prepared for sampling. One hundred milliliters of freshly prepared 2.5-per cent metaphosphoric acid solution were then placed in the clean dry bowl of the blendor. A representative sample of the food was weighed quickly to the nearest 0.1 gram and transferred immediately to the bowl of the blendor, containing the metaphosphoric acid and thoroughly blended. This process was generally completed in one to two minutes and was never prolonged to the point of heating of the mixture. When blending was complete, the material clinging to the sides was washed down by pouring the contents rapidly back and forth from a beaker until a uniform mix was obtained. Portions of this mixture were then centrifuged and aliquots taken for titration. Dilution of the aliquots was made with eight-per cent acetic acid, following the suggestion made by Musulin and King (1936) and practiced by others. In general, a determination was completed within less than 20 minutes from the time the food was prepared for weighing. Oranges, grapefruit, and other citrus fruits were sampled by expressing the juice by means of a glass reamer and filtering through cheesecloth. Portions of this filtered juice were then centrifuged and aliquots were diluted with the acetic acid for titration.

Loeffler and Ponting (1942) have suggested that for most fruits and berries the volume of insoluble solids can be neglected in the final calculation as being negligible, since the distribution of ascorbic acid is in the liquid phase, water plus soluble solids, rather than in the water phase only. In making calculations, this assumption was accepted for most of the fruits. For those foods for which it could not be accepted calculations were made on the basis of their water content, either as determined or as calculated from food tables.

Food Samples: Samples of the different foods tested were obtained in various ways. Some, including the oranges and grapefruits, were purchased from street venders, *vendedores ambulantes.* There was no way of determining the source of this material or the time it had been in transit. Other samples were obtained by purchase in the Río Piedras or San Juan markets, and here again the condition of the fruit was the only information available for recording. A few samples were gathered fortuitously by the author and these are noted. Still others were brought to the laboratory by members of the staff and presumably had not been long in transit. All determinations for fruits and vegetables were made on fresh raw products.

DESCRIPTION OF FOODS STUDIED AND METHOD OF SAMPLING

FRUITS [2]

Aguacate—Three fruits were used. The sample, analyzed 8/5/43, was purchased from a peddler; those of 8/29/43 were picked from a tree while still green and were analyzed after being kept in the laboratory until they were in an edible condition. The sample for analysis consisted of a narrow slice cut from stem end to blossom end with skin removed.

Caimito—One fruit was purchased from a peddler. The sample for analysis was a thin slice from stem end to blossom end and with seeds and skin removed.

[2] Used according to botanical interpretation.

China—All oranges were purchased from pushcart venders who go by the school each day from Santurce to San Juan. Practically all samples were made up of the composited juice from a dozen fruits.

China Mandarina—Fruits were purchased from various sources. The majority of the samples comprised the juice of six fruits.

Corazón—One fruit was purchased from a peddler. The sample for analysis was a narrow slice from stem end to blossom end with seeds and skin removed.

Frambuesa—A basket of fruit was purchased from a boy by the roadside near Barranquitas in the center of the island. The composited sample included seeds since these could not be removed.

Grocella—Several fruits were obtained from the supply for the diet kitchen of the hospital. For the sample the flesh of a number of fruits was removed from the seeds and composited.

Guanábana—Two fruits were purchased from a peddler. Both fruits were slightly overripe. The sample for analysis consisted of a slice at the center with skin and seeds removed.

Guayaba—(1) Four fruits were purchased from a peddler; two were pink fleshed and two were greenish. (2) The fruits were picked from a tree. Each sample consisted of parts from two to three fruits. The majority of the fruits were pink fleshed. (3) The fruit for this sample was also picked from a tree and was of the pink-fleshed variety. The samples for analysis were taken as a thin slice cut from stem end to blossom end and included skin and seeds.

Guineo—Several varieties of bananas were analyzed. All fruits were purchased in the market. A sample consisted of a crosswise slice taken near the middle of the peeled fruit. The sample of *manzanos* was slightly overripe, that of *rojos* in prime condition.

Jobo de la India—The fruits used were purchased from peddlers. All were fully ripe and firm. A sample consisted of a slice taken lengthwise of the fruit with the skin removed.

Lima—All fruits were brought to the laboratory by Dr. Ramón M. Suárez. They were small and varied from fully green to fully yellow. The majority of the analyses were on the composited juice of two to three fruits.

Limón de Sicilia—Each sample comprised the juice of three to four fruits.

Limón dulce—One fruit was brought to the laboratory by a member of the staff.

Mamey—One fruit was purchased from a peddler. The sample was a thin slice from stem end to blossom end with seeds and skin removed.

Mango—Fruits of the *mango pasote* and *mango piña* varieties were purchased in the Río Piedras market; those of the *mango de Mayagüez* were brought directly from Mayagüez to the laboratory. The fruits from the Virgin Islands were collected on a Saturday morning and analyzed the following Monday. Samples for analysis consisted of a thin slice cut from the fruit lengthwise after the skin had been removed.

Multa—A few fruits were brought to the laboratory and the sample was composited from several.

Naranja agria—One fruit was brought to the laboratory. The juice was expressed and treated for assay the same as the orange juice.

Níspola—The fruit was purchased from a peddler. The sample for analysis was a slice from stem end to blossom end with skin and seeds removed.

Panapén—The fruit used was purchased in the market. For the analysis a small section was cut near the center and trimmed to the edible part.

Papaya—All of the fruits used were presented to the laboratory. The ripe fruits had been carefully handled and were in prime condition. Sampling was done by cutting out a small section and trimming away the skin and seeds to the edible portion. Designations a, b, and c refer to sections taken from the stem end, middle, and blossom end, respectively. Fruit designated 2) was very ripe but in good condition.

Pepino—The fruits used were purchased in the market. Samples for analysis consisted of crosswise slices near the middle with the skin removed.

Pepino angolo—The fruit was brought to the laboratory. The sample was a crosswise slice near the middle with skin and seeds removed.

Piña—The fruit for analysis was purchased in the market. The value presented is an average for values obtained for a section of a slice near the top and one near the base.

Quenepa—The fruits used were purchased in the market. The flesh from 12 fruits was composited to make the sample for analysis.

Sapote—One fruit was purchased from a peddler. The sample for analysis was a thin slice cut away from the seed from stem end to blossom end with the skin removed.

Tamarindo—The fruits were brought to the laboratory. The flesh was removed from 12 or 15 and composited to give the sample for analysis.

Tomate del país—These fruits are from plants described as "grown from native seed." The outer surface is deeply creased and the fruits seldom reach a full red color. They do not have an attractive appearance and are used mostly for cooking. Samples for analysis consisted of wedged-shaped slices cut from stem end to blossom end. The skin was not removed.

Toronja—The grapefruit were purchased from the pushcart vender from whom the oranges were obtained. The juice for analysis was prepared in the same manner as the orange juice. Each sample comprised the juice of three to four grapefruit.

Uva de playa—The fruits used were brought to the laboratory. The pulp from several fruits was composited in the sample used for analysis.

STARCHY VEGETABLES

Batata—Two varieties of sweet potato were studied: (1) the common white-fleshed variety widely used in Puerto Rico and (2) the yellow-fleshed Puerto Rico Sweet, known on the continent as the Puerto Rico yam. A sample for analysis consisted of a slice near the center of a good-quality product.

Ñame de agua—One piece of root of medium diameter was purchased in the market. The sample for analysis consisted of a triangular wedge from a thin slice. The skin was removed.

Yautía blanca—Several *yautías* were purchased in the market. In preparation for sampling one well-shaped, medium-sized root was washed and the outer skin scraped. A thin wedge-shaped piece was then removed by cutting from crown to tap root end.

Yuca—A root of medium diameter was purchased in the market. The sample for analysis consisted of a wedge-shaped piece from a thin slice cut crosswise of the root. The skin was removed.

MISCELLANEOUS

Agua de coco—Fresh green coconuts were supplied by Dr. Ramón M. Suárez. An opening was made in the stem end of the fruit and aliquots of the liquid removed directly with a pipette.

Almendra—A number of immature nuts were taken from a tree, brought directly to the laboratory, and analyzed within a short time. The sample for analysis was composited from 11 nuts.

Caña de azúcar—A fresh sample of juice was prepared under the direction of Dr. Ramón M. Suárez and analyzed soon after it was brought to the laboratory.

Pasta de guayaba—1) was a commercial product made in Cuba. 2) was a commercial product made in Puerto Rico. 3)a was a product made by a Puerto Rican housewife. The pulp had been expressed from the raw crushed fruits and then cooked with sugar. Following the first determination the product was stored in the refrigerator and successive determinations—3)b, 3)c—were made at later dates. 4)a was a second lot of paste made by a Puerto Rican housewife. The paste had been prepared in the same way as product 3)a. An additional determination—4)b—was made after storage in the refrigerator. 5)a was a paste made from the same lot of guavas used for Lot 4)a, but the fruits were cooked before extracting the pulp. A corresponding analysis—5)b—was made on this product after storage in the refrigerator.

Jalea de guayaba—The sample used for analysis was from a commercial product made in Puerto Rico and was purchased in a local grocery.

RESULTS

Sixty-eight values for the ascorbic acid content of 40 food items produced or used in Puerto Rico are summarized (Table 1). In some cases the value for which the range is given is an average of several determinations, but in the majority of cases only one determination was made.

TABLE 1

Ascorbic Acid Content of Foods of Puerto Rico

Spanish name	English equivalent	Scientific name [1]	Date collected	Number samples	Ascorbic acid content	
					Range	Average
					mg./100 gm.	*mg./100 gm.*
Fruits						
Aguacate	Avocado	*Persea Persea* (L.)	8/5/43	1	.....	11.61
			8/29/43	2	15.64; 15.68	15.66
Caimito	Star apple	*Chrysophyllum Cainito* L.	4/20/43	1	.....	5.79
China	Orange	*Citrus sinensis* (L.) Osbeck	10/28/42-6/22/43	36	46.31-66.20[2]	56.95[2]
China mandarina	Tangerine	*Citrus nobilis* Lour.	11/10/42-12/1/42	4	22.75-35.65[2]	29.92
Corazón	Bullock's heart or custard apple	*Annona reticulata* L.	12/17/42	1	.....	34.53
Frambuesa (Fresa)	Mountain or wild raspberry	*Rubus rosaefolius*—Smith	3/7/43	1	.....	8.90
Grocella	Otaheite gooseberry	*Cicca disticha* L.	3/25/43	1	.....	7.54
Guanábana	Soursop	*Annona muricata* L.	11/23/42	2	26.78; 24.59	25.69
Guayaba	Guava	*Psidium guajava* L.				
1) Whole fruit			11/6-11/9/42	4	201.87-441.68	293.66
2) Whole fruit			4/13/43	4	210.02-259.76	234.58
Pulp only			4/13/43	4	226.82-280.40	253.30
3) Whole fruit			8/29/43	1	.....	149.10
Pulp only			8/29/43	1	.....	154.54
Guineo	Banana	*Musa sapientum* L.				
Gigante			11/18/42/ and 7/27/43	3	6.79-7.14	6.87
Enanos			7/27/43	1	.....	9.26
Manzanos			7/27/43	1	....	14.22
Niños			7/27/43	1	.....	9.60
Rojos			7/27/43	1	.....	5.58
Jobo de la India	Ambarella or golden apple	*Spondias dulcis*—Frost	11/16/42-12/21/42	9	45.44-63.78	50.08
Lima (Limón del pais)	Lime	*Citrus aurantifolia* (Christm) Swingle	11/4/42-11/20/42	10	23.10-38.09[2]	32.29[2]

Limón de Sicilia	Lemon	*Citrus limonia*—Osbeck	10/28/42-11/20/42	9	29.25-35.55[2]	32.29[2]
Limón dulce	Sweet lime	*Citrus limetta*—Risso	1/18/43	1	.....	26.33[2]
Mamey	Mamey or Mammee apple	*Mammea americana* L.	4/21/43	1	.....	10.86
Mango	Mango	*Mangifera indica* L.				
de Mayagüez			6/22/43	3	51.72-75.39	64.42
Pasote	Common variety		7/28 and 8/5/43	2	10.58; 9.13	9.86
Piña			8/4/43	2	45.88; 52.66	49.27
1) Common variety[3]			8/21/43	2	115.56; 121.42	118.49
2) Common variety[4]			8/21/43	1	.....	59.34
3) Common variety[5]			8/21/43	1	.....	79.94
Multa	Guava-Berry	*Eugenia floribunda*—West	9/4/43	1	.....	1.29
Naranja agria	Sour orange	*Citrus aurantium* L.	6/23/43	1	.....	73.04[2]
Níspola (Níspero)	Sapodilla	*Sapota Achras*—Mill	4/13/43	1	.....	20.02
Panapén	Breadfruit	*Artocarpus communis*	8/5/43	1	.....	35.32
Papaya	Papaya or papaw	*Carica papaya* L.				
1)a Verde	Green		6/21/43	1	.....	27.13
c Verde	Green					31.74
2)b Madura	Ripe		9/9/43	1	.....	108.56
3)a Madura	Ripe		9/25/43	1	.....	75.42
b Madura	Ripe					77.16
c Madura	Ripe					84.90
Pepino (Pepinillo)	Cucumber	*Cucumis sativus* L.	8/5/43 and 12/14/43	2	10.24; 5.91	8.08
Pepino angolo	Cassabana	*Sicania odorifera* (Vell) Naud	1/26/43	1	.....	20.78
Pimiento verde	Pepper, green, sweet	*Capsicum frutescens* L. *Capsicum annum* L.	11/16/42 and 11/25/42	4	45.94-96.01	73.10
Piña	Pineapple	*Ananas Ananas* (L.)	11/24/42	1	.....	14.84
Quenepa	Genip or Spanish lime	*Melicocca bijuga* L.	7/13/43	1	.....	6.68
Sapote o Zapote	Marmalade plum	*Manilkara duplicata* (Sesse and Moc.) Dubard	4/20/43	1	.....	46.79
Tamarindo	Tamarind	*Tamarindus indica* L.	3/25/43	1	.....	0.86
Tomate del pais	Tomato	*Lycopersycon Lycopersicon* (L.)—Karst	11/10-11/20/42 and 7/13/43	6	13.66-19.24	16.93

TABLE I *(Concluded)*

Spanish name	Spanish equivalent	Scientific name[1]	Date collected	Number samples	Ascorbic acid content	
					Range	Average
					mg./100 gm.	*mg./100 gm.*
Toronja	Grapefruit	*Citrus maxima* (Burm.) Merrill	11/4/42-4/13/43	14	35.60-52.88[2]	45.09[2]
Uva de playa (playera)	Sea grape	*Coccolobis uvifera* (L.) Jacq.	8/5/43	1	.....	0.48
Starchy vegetables						
Batata	Sweet potato	*Ipomoea batatas* L.)				
1) Common variety			11/25/42	1	.....	6.82
2) Puerto Rico Sweet	2) Puerto Rico yam		11/5/43	1	.....	17.32
Name de agua	White yam	*Dioscorea alata* L.	11/25/42	1	.....	9.51
Yautia blanca	White tanier	*Xanthosoma sagittae*—folium (L.) Schott	11/25/42	1	.....	10.02
Yuca	Cassava	*Manihot Manihot* (L.)	11/25/42	1	.....	33.77
Miscellaneous						
Agua de coco	Coconut water	*Cocos nucifera* L.	11/3/42-11/13/42	4	0.96-1.66[2]	1.35[2]
Almendra	Tropical almond	*Terminalia Catappa* L.	7/13/43	1	.....	3.60
Caña de azúcar jugo de	Sugar cane, juice	*Saccharum officinarum L.*	11/18/42	1	.....	0.48[2]
Pasta de Guayaba	Guava paste					
1)			4/13/43	1	.....	28.06
2)			6/22/43	1	.....	24.41
3)a			9/9/43	1	.....	74.88
3)b			10/13/43	1	.....	76.44
3)c			12/14/43	1	.....	70.68
4)a			10/13/43	1	.....	110.62
4)b			12/14/43	1	.....	91.72
5)a			10/13/43	1	.....	83.68
5)b			12/14/43	1	.....	69.64
Jalea de guayaba	Guava jelly		4/13/43	1	.....	4.42

[1] J. I. Otero y R. A. Toro, Catálogo de los Nombres Vulgares y Científicos de Alugnas Plantas Puertorriqueños. Bol. No. 37, Estación Experimental Insular, Departamento de Agricultura y Trabajo, Rio Piedras, P. R. [2] Values are per 100 milliliters. [3] Fruit picked from tree in Charlotte Amalie, St. Thomas, V. I.

All products, which would be classified botanically as fruits, have been put in one group regardless of whether they are described as fruits or vegetables from the standpoint of their use as foods.

A detailed summary of the values obtained for oranges and grapefruit samples, collected throughout the season, are presented (Table 2). Although it was never possible to determine from what part of the Island the fruits came, the array of values gives an idea of the uniformly high ascorbic acid content of oranges grown in Puerto Rico. The volume of juice per orange indicates the uniformity in the size of the fruit.

TABLE 2

Ascorbic Acid Content of Oranges and Grapefruit Grown in Puerto Rico

Date purchased	Orange juice			Grapefruit juice	
	Number fruits in sample	Ascorbic acid content	Volume juice per orange	Number fruits in sample	Ascorbic acid
		mg./ 100 ml.	*ml.*		*mg./ 100 ml.*
Oct. 28, 1942	8	64.58	....	....	.....
Nov. 3, 1942	12	60.11	....	....	.....
Nov. 4, 1942	12	64.49	110	3	52.83
Nov. 6, 1942	6	56.08	113	3	45.72
Nov. 9, 1942	12	54.30	119	3	48.32
Nov. 10, 1942	12	56.56	....	3	39.10
Nov. 13, 1942	12	49.80	105	3	35.60
Nov. 13, 1942	12	51.19	117	3	41.33
Nov. 18, 1942	12	49.83	118	3	52.88
Nov. 18, 1942	12	49.38	107	....	.....
Nov. 20, 1942	12	60.61[1]	103	....	.....
Nov. 23, 1942	12	47.94	132	....	.....
Nov. 25, 1942	12	62.89	123	4	49.47
Nov. 30, 1942	12	60.29	121	2	42.10
Nov. 30, 1942	9	65.45	110	....	.....
Nov. 30, 1942	12	56.14	112	....	.....
Dec. 14, 1942	12	54.29	88	....	.....
Dec. 15, 1942	12	53.45	93	....	.....
Dec. 21, 1942	12	49.30	102	(12/29)	.....
Dec. 28, 1942	6	47.59	112	4	45.38
Dec. 30, 1942	12	57.31	87	....	.....
Jan. 5, 1943	12	59.63	112	....	.....
Jan. 13, 1943	12	55.79	120	....	.....
Jan. 18, 1943	12	54.42	105	....	.....
Jan. 25, 1943	12	46.31	134	....	.....
Jan. 28, 1943	6	61.46	101	....	.....
Feb. 1, 1943	12	66.20	101	....	.....
Feb. 8, 1943	12	61.14	....	....	
Feb. 15, 1943	12	61.88	104	3	43.73
Feb. 23, 1943	12	64.54	100	3	47.51
Mar. 3, 1943	12	58.95	83	....	.....
Mar. 11, 1943	12	59.39	92	3	46.85
Mar. 15, 1943	12	56.89	....	(4/13)	.....
April 14, 1943	6	47.35	115	4	40.40
April 27, 1943	10	61.00	55	....	.....
June 22, 1943	10	63.84	60	....	.....
		(Av.) 56.95			(Av.) 45.09

[1] From Mayagüez.

DISCUSSION AND SUMMARY

As a consequence of the low intake of vitamin C-rich foods in Puerto Rico, indicated by a study of plasma ascorbic acid concentration for groups of individuals of widely different economic levels, a study was undertaken to determine the vitamin C content of foods grown on the Island that might be used more extensively in the diet as a source of this important vitamin.

Samples of 40 food products were studied; these included 31 true fruits, four starchy vegetables, and five miscellaneous products. The list of fruits included several that are eaten as vegetables either cooked or raw. The foods studied were selected on the basis of a probable significantly high ascorbic acid content and, in addition, because they are produced extensively on the Island or may be produced readily and in abundance.

The highest values were obtained with guavas, values ranging from 149.10 milligrams per 100 grams of whole fruit to 441.68 milligrams. Values are reported on the basis of pulp free of seeds as well as for the whole fruit (Table 1). When the fruit is eaten raw, the skin and seeds are included but the seeds and much of the skin are removed in making the paste.

The average value of 56.95 milligrams per 100 milliliters of orange juice is well above the average generally given in food tables. Furthermore, this high concentration remained relatively uniform throughout the season. The volume of juice per orange indicated that one orange per day will supply a satisfactory amount of vitamin C. During most of the season oranges of the size used may be purchased for one cent each, indicating the low cost of an adequate supply of vitamin C during the orange season.

The value of 45.09 milligrams for grapefruit juice and 32.29 milligrams for lime juice and lemon juice are similar to average values generally accepted.

The values for the several varieties of mangoes showed wide variations, ranging from a value of 9.86 milligrams for the *mango pasote*, or the common variety of Puerto Rico, to 118.49 milligrams for the so-called "common variety" obtained in St. Thomas, Virgin Islands. Mangoes grow readily in Puerto Rico and should be grown more extensively, especially the better varieties.

The papaya, another fruit that can be grown in practically all sections of the island, gave high values of 75.42 to 108.56 milligrams for the ripe fruit. The unripe fruit contained considerably less, 27.13 to 31.74 milligrams.

Avocados gave values of 11.61 and 15.66 milligrams. These fruits are produced in relative abundance and are relished by those who can afford to buy them.

Other fruits having a relatively high ascorbic acid value, which may be produced and used more extensively, are the *Corazón* (Bullock's heart or custard apple) with a value of 34.53 milligrams; the *Guanábana* (soursop), 25.69 milligrams; the *Jobo de la India* (ambarella or golden apple), 50.08 milligrams; the *Níspola* (sapodilla), 20.22 milligrams; and the *Sapote* (marmalade plum), 46.79 milligrams.

Pineapples are extensively cultivated in Puerto Rico. In former times they were grown primarily for the export trade but at present the crop is available for consumption locally. The variety studied has an excellent flavor and its value of 14.84 milligrams per 100 grams makes it a significant food for vitamin C.

The four starchy vegetables studied are the ones used most extensively. The value of 6.82 milligrams for the white-fleshed sweetpotato does not make this variety significant for vitamin C. The higher value of 17.32 milligrams for the yellow-fleshed variety gives another reason, in addition to the vitamin A content, for using the yellow varieties in preference to the white. *Ñame, yautía,* and *yuca* are of significance as sources of vitamin C only if cooked so as to conserve this vitamin. This is not usually done in Puerto Rico any more than is the case with the cooking of similar vegetables elsewhere.

The tropical almond grows wild over the entire island. Green nuts are on the trees at practically all times of the year and the meats are relished by young boys. The results for vitamin C content reported here indicate that whatever other food values the nuts may have they cannot be considered important for vitamin C.

The values for home-prepared guava paste, ranging from 74.88 to 110.62 milligrams for the paste prepared from uncooked fruit, give a basis for making this food product an important source of vitamin C in the diet.

With the abundance of fruits with a high content of vitamin C that are grown and can be grown in Puerto Rico at all times of the year, there seems little excuse for anyone consuming a diet that does not contain an abundance of this vitamin.

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DIGESTIBILITY OF MILK AS AFFECTED BY VARIOUS TYPES OF TREATMENT

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Numerous methods have been used to demonstrate variations in the digestibility of various types of milk. The physical methods of testing proposed by Alleman and Schmid (1916); Hill (1923); Chambers, Wolman, and Hands (1939); and Doan and Dizikes (1942) in general measure the amount of resistance the milk curds offer to penetration or fracture under standard conditions of temperature, acidity, concentration of enzymes added, agitation, and pressure.

In vivo measurements of milk digestibility have been reported by Espe and Dye (1932); Doan and Flora (1939); Reynolds, Macy, and Souders (1939); Sommer (1942); Hess, Koch, and Sennewald (1926); Conquest, Turner, and Reynolds (1938); Hess, Poncher, and Woodward (1934); Hess, Poncher, Wade, and Ricewasser (1940); Fetter and Schultz (1935); and Nevens and Shaw (1933).

The *in vitro* techniques which have been employed by Fetter and Schultz (1935); Doan and Dizikes (1942); Doan and Flora (1939); Hess, Koch, and Sennewald (1926); Hull (1938); and Wallen-Lawrence and Koch (1930) suggest the possibility of using methods of testing that are convenient and rapid.

A study of these investigations reveals that the physical methods employed do not necessarily measure milk digestibility since they rely almost entirely upon the curdling characteristics of the milk. The *in vivo* studies involve uncontrolled factors and are time consuming. The studies involving the use of the *in vitro* digestion techniques mentioned above, approximate the conditions of digestion known to exist in the human stomach and intestines with the exception that they do not involve the use of as wide a variety of enzymes as are present in the gastrointestinal tract during digestion. In many of the *in vitro* studies which were examined the milk proteins were digested to the amino acid stage and it was believed that under these conditions the most important part of the digestion picture—the disintegration of the larger protein particles—was not observed.

EXPERIMENTAL PROCEDURE

In order to eliminate the disadvantages of the *in vitro* digestion methods a digestibility test was devised which included the use of both acid and alkaline digestion periods in the presence of enzymes from the hog stomach, duodenum, and pancreas. The digestion of the larger protein particles, or the acid-insoluble (pH 4.7) protein fraction, was observed by measuring its rate of conversion to acid-soluble (pH 4.7) protein. This was accomplished by adjusting the pH of samples taken from the digest at specified

intervals to 4.7, filtering, and determining the total Kjeldahl nitrogen content of the filtrate. The first 30 minutes of digestion was carried out at pH 4.7 so that samples removed from the digest during this period were filtered directly. The difference between the total protein content of the milk and the amount of milk protein which was soluble at pH 4.7 prior to digestion was designated the acid-insoluble protein content of the milk.

The rate of digestion of the acid-insoluble milk proteins was used as the index of milk digestibility in preference to using the rate of fat or sugar digestion because it has been demonstrated that it is the large insoluble casein curds which form from cow's milk that are largely responsible for the gastrointestinal disturbances of infants fed pasteurized or raw, whole cow's milk, Brennemann (1913) and Hill (1923).

In measuring the digestibility of milk it was found that the type and amount of digestive enzymes used were of extreme importance. The first trials using this method were not successful because the amount of gastric and pancreatic-duodenal enzymes employed during digestion were too large. It was only after using very small amounts of each that differences in the digestion rates became apparent.

The gastric enzymes used were those present in U.S.P. pepsin. The pancreatic-duodenal enzymes used were those prepared by mixing one part of macerated hog duodenum with 10 parts of macerated hog pancreas, drying the mixture *in vacuo*, defatting the dried material with a hydrocarbon fat solvent, pulverizing the defatted product and finally standardizing the powder by dilution with starch to 30 U.S.P. trypsin units per gram of powder by the Fuld-Gross method of assay for trypsin. Sometimes U.S.P. trypsin (pancreatin) is prepared in this manner. The enzymes used throughout the investigation were stored at 5°C.(41°F.) in an atmosphere of low moisture content to prevent changes in potency.

Three to 10 trials were made on each type of milk tested in the interest of accuracy. All results were compared with those of pasteurized whole milk, which was used as the control substance throughout the investigation.

A description of the technique which was adopted is as follows: 800 ml. of milk at 37°C.(98.6°F.) was mixed with 80 ml. of 0.5 N HCl containing 0.06 per cent gastric enzymes by rapidly pouring the milk into the acid and then stirring vigorously. The resulting pH of the mixture was always 4.7 ± 1. Fifty-ml. portions of digest were removed and filtered after five and 30 minutes of mild agitation and digestion at 37°C. The filtration of the sample was completed within one minute so that the digestion occurring during filtration did not materially alter the soluble-protein content of the filtrates. The filtrates were set aside for total-protein analysis. The digest was then adjusted to pH 7.5 with NaOH dropwise and with constant stirring. Five minutes after the NaOH had been added, 0.125 gram of the pancreatic-duodenal enzymes described above was added to the digestion mixture. After 15 minutes of mild agitation at 37°C. a third 50-ml. portion was removed and adjusted to pH 4.7 with about three ml. of N-HCl and then filtered to remove the acid-insoluble protein from the digest sample. Similarly, 50-ml. portions of the digest were adjusted to pH 4.7 and filtered after 70, 90, 120, 150, and 180 minutes of digestion. The acid

inhibited further pancreatic-duodenal digestion during filtration. The acidification of each sample was done rapidly to minimize the effect of time on the acid-insoluble protein content of the digest sample. During the alkaline digestion a pH level of 7.5 ± 0.1 was maintained by the periodic addition of NaOH. A record was kept of the volume of HCl and NaOH solutions which were added to the digest so that the proper dilution factor could be used in the calculations.

Some precautions to be observed in using the above method are as follows:

1. The enzymes must be accurately weighed out on an analytical balance.
2. The addition of alkali to the digest must be done dropwise with constant agitation.
3. The alkaline part of the digestion must be maintained at pH 7.5 ± 0.1.
4. Five minutes must be allowed for the complete precipitation at pH 4.7 of the acid-insoluble proteins in the digest sample.
5. The temperature of the digest must be maintained at 37°C.(98.6°F.).
6. The milk must be poured into the acid as rapidly as possible.
7. If the same batch of enzymes cannot be used throughout a series of tests the new batches must be assayed carefully so that comparable amounts of enzymes are present in the digest.

The above technique was used to study the relative digestibility of the following types of milk:

A. Cow's milks which were not heated to a temperature higher than 65°C.(149°F.).

1. Raw whole milk.
2. Whole milk pasteurized at 62.5°C.(144.5°F.) for 30 minutes.
3. Homogenized whole milk—Berry (1934).
4. Zeolite-treated whole milk—Lyman, Browne, and Otting (1933).
5. Enzyme-treated whole milk—Conquest, Turner, and Reynolds (1938).
6. Skimmed milk pasteurized at 62.5°C.(144.5°F.) for 30 minutes.
7. Pasteurized skimmed milk dried on a roller-vacuum drier at 48.5°C.(119.3°F.).
8. Pasteurized and enzyme-treated whole milk each containing 20 per cent $BaSO_4$, intended for X-ray purposes.

B. Cow's milks which were heated to at least 87.5°C. (189.5°F.) some time during processing, designated hyper-heated milks for purposes of this report.

1. Pasteurized whole milk brought to a boil on an electric hot plate and cooled immediately in cold water to 10°C.(50°F.).
2. Pasteurized, homogenized whole milk boiled as above.
3. Pasteurized, zeolite-treated whole milk boiled as above.
4. Pasteurized, enzyme-treated whole milk boiled as above.
5. Pasteurized whole milk autoclaved for 20 minutes at 15 pounds pressure.
6. Pasteurized, enzyme-treated whole milk autoclaved as above.
7. Whole milk evaporated to 26 per cent solids previously preheated to 90°C. (194°F.) and sterilized in eight-ounce tin cans for 45 minutes at 15 pounds steam pressure and reconstituted with distilled water.
8. Evaporated whole milk prepared as above, containing 0.1 per cent Na_2HPO_4, and reconstituted with distilled water.
9. Evaporated whole milk containing 0.1 per cent Na_2HPO_4 prepared as above, having been treated with one gram of U.S.P. trypsin in each 5,000 grams of cold evaporated milk immediately prior to canning and sterilizing at 15 pounds pressure for 45 minutes, and reconstituted with distilled water.
10. Sweetened, condensed whole milk pasteurized at 87.5°C.(189.5°F.) for one hour and reconstituted to its original volume with distilled water.
11. Roller vacuum-dried skim milk preheated to 90°C.(194°F.) for three minutes prior to drying and reconstituted with distilled water.
12. Spray-dried whole milk preheated to 90°C.(194°F.) for seven minutes before drying and reconstituted with distilled water.

C. Milk from other species:
1. Human milk.
2. Mare's milk.
3. Goat's milk.

The Babcock method for determining the fat content of milk was used throughout this investigation. All protein assays reported are the product of the macro-Kjeldahl nitrogen content of the sample and the factor 6.38. The pH determinations were made with a glass electrode and a mecury-calomel-KCl cell. Curd tension measurements were made according to the recommendations of the American Dairy Science Association in the Report of the Committee on Curd Tension (1938).

RESULTS AND DISCUSSION

Results obtained in applying the digestibility test to the various types of milk listed above are presented (Table 1); the data have been condensed so that only the significant variations encountered are demonstrated. The results are expressed as the average per cent of acid-insoluble protein that was converted to acid-soluble protein during digestion. Those milks showing a higher percentage of conversion than that obtained in testing pasteurized, whole cow's milk were considered as having superior relative digestibilities and vice versa.

In the group of cow's milks which were not hyper-heated only those that were enzyme-treated or mixed with barium sulfate demonstrated significant variations in their relative digestibilities.

After 180 minutes of digestion 61.2 per cent of the acid-insoluble protein present in enzyme-treated milk was found to be acid-soluble as compared with 48.4 per cent for pasteurized whole milk. In a preliminary report Turner (1941) found that casein prepared from enzyme-treated milk was changed chemically by the enzyme treatment. It is believed that these observed changes are responsible for the improved relative digestibility of enzyme-treated milk.

Hyper-heated milks contain a low acid-soluble protein content before digestion. In spite of this fact 52.3 per cent of their acid-insoluble proteins were converted to acid-soluble proteins during the digestibility test. It is felt that these findings demonstrate the improved digestibility of hyper-heated milks. The results of the digestibility test on boiled milk are shown in the table. All hyper-heated milks which were not enzyme-treated digested similarly. The enzyme-treated, hyper-heated milks digested slightly better than hyper-heated milks.

Pasteurization, skimming, homogenization, desiccation, and zeolite treatment had no significant effect on the relative digestibility of cow's milk throughout the investigation. Dried milks which were hyper-heated prior to drying digested similar to all other hyper-heated milks, and dried milks not hyper-heated digested similar to all other milks not hyper-heated.

It appears that the favorable results which have been reported in feeding homogenized and zeolite-treated milk to infants may be due to the fact that large curds, which could mechanically irritate an infant's stomach, do not form from these milks.

Reynolds, Macy, and Souders (1939) and Sommer (1942) fed test meals containing 33 and 20 per cent barium sulfate, respectively, in hard and

soft curd milks to children. They observed the gastrointestinal motility of these mixtures roentgenologically.

Reynolds *et al.* concluded that soft curd milks leave the stomach more rapidly than hard curd milks. They state that their results are in agreement with the favorable metabolic and clinical observations obtained when feeding soft curd milks to children.

TABLE 1

Relative Digestibility of Various Types of Milk[1]

Time of digestion	Whole cow's milk					Milk from other species		
	Milk not hyper-heated			Hyper-heated milk				
	Pasteurized	Enzyme treated	Pasteurized plus $BaSO_4$	Boiled	Boiled, enzyme treated	Human	Mare	Goat
min.								
5	0	0	0	0	0	0	0	0
30	3.5	4.6	2.8	6.3	5.5	7.8	7.2	5.3
50	10.0	20.1	7.4	13.4	13.5	15.6	19.5	14.6
70	16.1	25.2	11.0	19.4	19.8	27.4	30.9	18.5
90	23.6	30.3	13.9	27.3	28.1	35.3	37.1	22.1
120	34.5	39.2	18.0	34.3	35.7	47.0	47.4	26.9
150	43.6	50.9	23.7	42.5	45.6	60.7	51.5	31.4
180	48.4	61.2	30.6	52.3	53.9	84.3	59.7	40.8
Curd tension (gm.)..	41	18	51	5	2	0	.....	27
Fat in milk (pct.)....	3.50	3.50	3.50	3.50	3.50	3.60	.....	4.20
Protein in milk (pct.).....	3.01	3.01	3.12	3.12	3.10	0.98	1.97	3.20
Acid-soluble protein in 100 ml. milk before digestion (gm.).....	0.72	0.87	0.68	0.44	0.58	0.47	1.00	0.94

[1]Average per cent of acid-insoluble milk protein converted to acid-soluble protein during digestion.

Sommer reached a different conclusion. He submitted his data to statistical analysis and found that there was no statistically significant difference in stomach-emptying time for milks of different curd tension fed to children. He also found that when the data presented by Reynolds *et al.* were statistically analyzed, their work showed no significant difference in the stomach-emptying time for milks of different curd tension. Sommer also demonstrated that barium sulfate raised the curd tension of the milks he studied.

The effect of barium sulfate on the curd tension and relative digestibility of pasteurized whole milk (Table 1) shows that an average of only 30.6 per cent of the acid-insoluble protein of pasteurized milk containing 20 per cent barium sulfate was converted to acid-soluble protein at the end of the digestion period.

The gel structure of curds containing 20 per cent barium sulfate formed from milk during the curd tension test was remarkably weak. It was found that mild agitation disintegrated them to approximately the same fine state of subdivision regardless of whether or not they were formed from milks of different curd tension.

It is suggested that the increased curd tension of milk containing 20 per cent barium sulfate is due to the frictional resistance of barium sulfate and its adsorbed materials on the knife of the curdometer.

The above results indicate that the negative findings of Sommer may be due in part to the fact that barium sulfate tends to equalize the gastrointestinal motilities of milks of different curd tension.

Goat's milk did not digest as readily as cow's milk. Its curd tension was relatively low. It was not necessary to change the digestibility test when applying it to goat's milk.

Mare's milk and human milk were found to be buffered much less than cow's milk and goat's milk. It was necessary to use much less acid in adjusting the pH of human and mare's milk to 4.7. However, in order to keep the dilution factor the same for all the milks studied, the volume of acid and alkali added to the digest was kept constant throughout. The amount of enzymes used to digest each 100 ml. of milk was never changed regardless of species.

Human milk is known to be more readily tolerated by infants than cow's milk, Brennemann (1913) and Hill (1923). It appears that the data shown in the table substantiate this fact since there is so large a part (84.3 per cent) of the acid-insoluble protein in human milk converted to acid-soluble protein by the end of the digestion period. Other factors that help explain the superior digestibility and adaptability of human milk to the infant are its low total-protein content and its proportionately high acid-soluble protein content before digestion. Both of these factors should aid in preventing the formation of large hard curds that would mechanically irritate the infant's stomach.

Mare's milk, in spite of its low total-protein content, was found to contain much more acid-soluble protein prior to digestion than was found in cow's milk. The percentage of acid-insoluble protein that had been converted to acid-soluble protein at the end of the digestion period was 59.7 per cent, which is significantly greater than that found in cow's milk but less than the amount found in human milk.

It is believed that the digestibility test described and used in this investigation is capable of revealing differences in the relative digestibilities of various types of milk. This is brought out by the fact that the data obtained in using the test can be correlated with other factors concerned in milk digestion, i.e., low-protein milks tend to digest more readily than high-protein milks; the well-known superior digestibility of human milk is substantiated by the results reported; those milks in which demonstrable chemical changes in the protein occurs, such as is found in enzyme-treated and hyper-heated milk, are also more easily digested.

SUMMARY

The *in vitro* digestibility test employed in this study appears to be a satisfactory procedure to use in demonstrating differences in the relative digestibility of various types of milk.

Pasteurization, homogenization, desiccation, zeolite treatment, and skimming did not appreciably alter the relative digestibility of cow's milk.

Enzyme-treated and hyper-heated milks digested relatively faster than pasteurized whole cow's milk.

The fact that human milk is more satisfactory for infant feeding than cow's milk has been substantiated.

Barium sulfate prevented the normal digestion of milk protein and weakened the gel structure of milk curds under the conditions of this investigation.

Mare's milk digested more readily than cow's milk and goat's milk.

The relative digestibility of goat's milk was found to be inferior to that of pasteurized whole cow's milk.

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DETERMINATION OF SOYBEAN FLOUR IN MEAT PRODUCTS

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Meat food products, such as sausage and meat loaves, are made from finely ground meat highly seasoned with various spices and may be stuffed into casings made from cellophane or the cleaned and prepared intestine skin of cattle, sheep, and hogs. Some such products are made from cured meat, smoked and cooked. Bureau of Animal Industry, Order 211-revised, provides that the combined amount of cereal, starch, flour, and milk powder in sausages may not exceed five per cent, while meat loaves, tripe with sauce, stew, hash, chili con carne, tamales, scrapple, and the like may contain flour and other substances in larger amounts. Soybean flour is finding a use in such products, especially those combinations of inferior cuts called "artificial bologna," cottage loaves," and the like. It is becoming increasingly obvious that eventually a satisfactory method of analysis for determining the percentage of soy flour must be developed.

There are several available methods at present. These are based upon some particular constituent of the soybean which is distinct for that product and will differentiate it from the many other ingredients which may be added to the meat products. It is necessary that this constituent must withstand the cooking and smoking procedures sometimes used in the manufacture of sausage.

La Wall and Harrison (1934) presented a test based on the enzyme urease which may be detected by the liberation of ammonia from urea. This test is unsatisfactory in those cases where the urease is completely or partially destroyed by the temperature of cooking or smoking. The same workers advised confirming the test by a microscopic examination and determining the presence of the characteristic I-shaped or hourglass cells. Modern milling methods tend to reduce the number of these cells present, but in all specimens examined by our laboratory it has been possible to find them by concentration methods. In any case these tests are bound to be only qualitative.

Hendrey (1939) proposed a method based on the determination of insoluble, nonfermentable sugars. In his procedure the moisture and fat are removed from the product, the soluble sugars are removed with 50 per cent alcohol, and the remaining carbohydrates are hydrolyzed with acid and then fermented with yeast. The sugars remaining after fermentation are determined and multiplied by a factor. Lythgoe, Ferguson, and Racicot (1941) investigated this method and decided it was sufficiently accurate for law enforcement purposes but not specific for soybean content.

Hayward (1939) reported a method based on the nitrogen-free extract in meat, but in his method the presence of liver is liable to cause variable results.

Bailey (1942) of the Illinois Department of Agriculture, probably has developed the best chemical method reported to date. This method is based on the fact that the chief protein of soybean is a globulin, glycinin, according to the work of Osborne and Campbell (1900). Bailey separates the glycinin from the other proteins found in meats and vegetable substances added to meats. Glycinin can be boiled in a water solution with little or no coagulation and the remaining soluble portion can still be precipitated at its iso-electric point. On the other hand, the albumins and animal globulins are coagulated by heat. By redissolving the glycinin in 10 per cent sodium chloride and saturating it with magnesium sulfate and boiling, gelatin and other soluble meat proteins are precipitated. Further saturation with sodium sulfate finally precipitates the glycinin, which can be purified by dissolving this precipitate and precipitating with half ammonium-sulfate saturation. This purified glycinin can again be dissolved, precipitated as an insoluble metal proteinate, thoroughly washed, and weighed. Thus in nine or 10 steps the copper proteinate is weighed and the percentage of soy flour present can be calculated accurately according to a formula. Although the method is somewhat time consuming, this is the only criticism possible as there is no need for special apparatus or special skill.

There is another important method, suggested by Glynn (1939) and further investigated by Ferguson, Racicot, and Rane (1942), based on the zone phenomenon produced in the constant antibody, optimal antigen-antibody ratio described by Dean and Webb (1926). This method is specific for soybeans and in our laboratory has given accurate and reproducible results. Since our adaptation of this method varies somewhat from the original work of Ferguson *et al.*, it is thought advisable to report our work in detail.

EXPERIMENTAL PROCEDURE

For the preparation of the antiserum we used Coca's solution to prepare an extract of the soy flour.

Coca's Solution

Sodium chloride	5 grams
Sodium bicarbonate	2.75 grams
Phenol	4 grams
Distilled water	1,000 ml.

The sodium chloride extract, as used by Ferguson, we found to be somewhat low in soy protein, while the extract made with two per cent urea, as proposed by them, contained such a high percentage of nonprotein nitrogen that we decided against its use. Ten grams of soy flour were mixed with 100 ml. of Coca's solution and shaken in a mechanical shaker for one hour. The mixture was allowed to stand overnight and was shaken for one hour the following day. After the second shaking the mixture was centrifuged at high speed and the supernatant fluid filtered through paper. It was bottled in sterile 60-ml. vials and pasteurized twice on successive days at 60°C.(140°F.) for one hour. While this heating process did not destroy all the sporeforming organisms present, the extract was found to be satisfactory. It is believed that certain sporeformers survive the heating

process because the phenol present in the extracting medium did not facilitate a return to the vegetative stage until a reduced concentration was reached in the cultivation medium.

Protein Nitrogen Content of Extracts

Lot 1—312 mg. protein nitrogen per 100 ml.
Lot 2—290 mg. protein nitrogen per 100 ml.
Lot 3—346 mg. protein nitrogen per 100 ml.
Lot 4—265 mg. protein nitrogen per 100 ml.
Lot 5—355 mg. protein nitrogen per 100 ml.
Lot 6—287 mg. protein nitrogen per 100 ml.
N.P.N. was very low in these extracts.

No allowance was made in the rabbit injections for the variation in these extracts.

Injections were all made intraperitoneally at five-day intervals starting with two ml. and increasing by two ml. until a dose of 12 ml. was reached. At this time the rabbits were given a rest from injections for three weeks and another series of injections was given exactly as before. Five days after the end of the second series of injections the rabbits were bled, an average of 25 ml. of blood being taken from each rabbit. From then on the process was continuous. Five days after bleeding 12 ml. of the extract were injected and five days later the rabbits were bled again. In this way a goodly supply of antiserum was produced. The strength or titer of the serum in terms of soybean flour is based on the method proposed by Glynn (1939). The actual titration is performed as follows:

Grind 25 grams of sausage, known to be free from soybean flour, add 0.25 gram of soybean flour and 100 ml. of five-per cent sodium chloride solution, and shake in a machine for one hour. Filter the extract through paper. Set up a rack of 10 Wassermann tubes having the following dilutions:

1:450	1:675	1:900	1:1,350	1:1,800	1:2,700	1:3,600	1:5,400	1:7,200	1:10,800

The undiluted extract is calculated as 1:450 by taking into consideration the fact that the sausage is about 50 per cent moisture. Thus 25 grams of meat would yield 12.5 ml. of fluid. This added to 100 ml. equals a dilution of 112.5 ml. to 0.25 gram of flour or a dilution of 1:450. Each tube contains one ml. of the indicated dilution.

A definite amount of serum was added to each tube. The tubes were shaken well and incubated in a 56°C.(132.8°F.) water bath for two and one-half hours. In the first titration we used 0.5 ml. serum. This gave a positive flocculation in Tubes 1 to 8, inclusive. Obviously, the ratio of antibody to antigen was not suitable to obtain a proper zone. When the amount of serum added to each tube was reduced to 0.2 ml., an excellent zone was obtained. The first two tubes remained cloudy and the last two tubes were cloudy with distinct flocculation in the third and eighth tubes, inclusive. By adjusting the amount of serum, concentrating the serum, or diluting the extract a zone can be obtained to suit any percentage of soy flour present.

In such a series, the tube farthest to the left showing distinct flocculation is the "indicator" tube, or the one which indicates the titer of the

serum. Since the amount of soy flour added to sausage will be in multiples of one per cent once the titer of a serum is established, it is only necessary to divide the dilution of the indicator tube for the unknown sample by that of the indicator tube for the serum titration and the result will be the percentage of added flour. For example, if the third tube is the furthest tube to the left showing flocculation in the original titration of the serum and an unknown sample is set up exactly as if it contained one per cent of soy flour and shows the furthest tube to the left to be the fifth tube, then the dilution of the fifth tube (1-1,800) divided by the dilution of the third tube (1-900) will give a result showing two per cent of soy flour added to the product.

Investigations were made in an effort to determine if phenolization of the serum would interfere with the test, if the keeping qualities of the serum would be improved, or if the titer of the serum would change over a period of time. A 7.5 per cent solution of phenol was used and 7.15 ml. of this solution were added to 100 ml. of serum giving a strength of 0.5 per cent phenol in the final serum. A preliminary titration of the phenolized and nonphenolized portions gave identical results. Later more serum was added and the entire lot phenolized. Owing to the inclusion of lower strength serum the titration was changed but a very satisfactory titration was made using various amounts of added soy flour. Sufficient time has not elapsed to show how long this serum will retain its original titer.

In an effort to hasten reaction time for this test, the serum was concentrated. The concentration was performed in two different ways. One lot of 60 ml. of the phenolized serum was put in a sterile cellophane sausage casing and placed in a refrigerator with a small fan behind it. Within 40 hours the amount of serum had been reduced from 60 to 34 ml.

In the other method of concentration, 15 ml. of serum was placed in a sterile container and frozen with dry ice. A low vacuum was applied for eight hours and the volume was reduced to 10 ml.

This concentrated serum gave a much prompter response in that the reaction was completed in 45 minutes as compared with two and one-half hours for the unconcentrated serum. However, the zone was shifted to the left, indicating that the antibody-antigen relation of this titration was not satisfactory.

In view of the zone shift with concentrated serum, it was decided to use our serum as produced and phenolized. The zone appeared in the proper place and would permit the determination of as little as 0.5 per cent soybean flour. In cases where more than five per cent of soybean flour is added to a product, it is necessary to prepare the unknown extract as if it contained one per cent soy flour and then dilute it 1 + 3 with distilled water. After the determination the result will of course be multiplied by four.

DISCUSSION

In applying this test to an unknown sample it is suggested that a known one-per cent extract be made and run in parallel with the unknown specimen made up as if it contained one per cent of flour. If the indicator tube

of the known one-per cent extract is in the second to the fifth tube and no reaction takes place in the extract from the unknown sample, it means that the unknown contains less than 0.5 per cent soy flour or more than five per cent. In such a case it is advisable to dilute the extract of the unknown 1 + 3 with distilled water and repeat. Determine the percentage of soy flour as shown by the diluted extract and multiply by the dilution.

A Typical Test of Serum

Soy flour added	Dilutions									
	1/450	1/625	1/900	1/1,350	1/1,800	1/2,700	1/3,600	1/5,400	1/7,200	1/10,800
pct.										
1	.....	.....	++++	++++	++++	.....	.....	.....	.....	.....
2	.....	.....	.....	.....	++++	++++	++++	.....	.....	.....
3	.....	.....	.....	.....	.....	++++	++++	.....	.....	.....
4	.....	.....	.....	.....	.....	++	++++	++++	.....	.....
5	.....	.....	.....	.....	.....	.....	.....	++++	++++	.....

With the single exception that a small amount of flocculation appeared in the sixth tube in the four-per cent extract, this test revealed a suitable serum which was exact in nearly all strengths of extract.

Tests made on commercial products produced results which checked exactly with the amount of soybean flour as indicated by the manufacturers' formulas.

Five varieties of soybean flour were used in our investigations which contained from 42 to 52 per cent protein. Since chemical and serological methods both use the protein factor as an indication of the amount of total flour and since the total percentage does not vary more than 10 per cent, this factor is not considered a serious disadvantage in the use of either test. To be absolutely accurate for either test it would be necessary to standardize both methods against the soybean flour being used. A distinct disadvantage of this method is the time required for the production of a suitable serum. If a large number of determinations are to be made, however, a supply of the serum can be continuously produced with little effort.

SUMMARY

A satisfactory method for the determination of the amount of soybean flour in meat products is necessary.

Reference is made to a satisfactory chemical method.

A serological method is described in detail.

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CONSTITUENTS OF THE CRUDE CAROTENE OF CERTAIN HUMAN FOODS

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Some years ago Shinn, Kane, Wiseman, and Cary (1937) pointed out that the carotene obtained by the analytical methods in use at that time contained impurities. Various methods for purifying the crude carotene were proposed by Fraps and Kemmerer (1939); Hegsted, Porter, and Peterson (1939); Moore (1940); Kemmerer (1941); Wall and Kelly (1943); Haagen-Smit, Jeffreys, and Kirchner (1943). Recently Beadle and Zscheile (1942) showed that the carotene extracts of spinach and other fresh vegetables contained, besides beta carotene, neo-beta-carotene. Kemmerer and Fraps (1943) found that the carotene obtained by all the methods referred to above contained impurity A, carotenoid X, and the neo-beta-carotene of Beadle and Zscheile (1942) as well as beta carotene and sometimes alpha carotene and neo-alpha-carotene. Kemmerer and Fraps (1944) showed that the carotenoid X was identical with neo-beta-carotene U. Neo-beta-carotene U and B had previously been prepared and shown to be stereoisomers of beta-carotene by Polgar and Zechmeister (1942). According to Kemmerer and Fraps (1943) neo-β-carotene U has no vitamin A activity and neo-β-carotene B has one-half the activity of β-carotene.

The carotene contents of some fresh and frozen green vegetables have been determined by Zscheile, Beadle, and Kraybill (1943). They used a spectrophotometric method and assumed that the principal pigments in the extracts were beta-carotene and neo-beta-carotene. Since most vegetables also contain neo-β-carotene U, and a few contain alpha carotene, it seemed important to ascertain the relative amounts of the different carotene pigments in various vegetables and to express their content of vitamin A-active pigments in terms of a single unit. In this paper the term beta-carotene equivalent is used and is defined as the quantity of the beta-carotene plus one-half the neo-β-carotene B plus one-half the alpha carotene. The latter two carotenes have half the vitamin A potency of beta-carotene.

EXPERIMENTAL PROCEDURE

The crude carotene extracts of the vegetables were subjected to chromatographic analysis by a method previously described by Kemmerer and Fraps (1943). The procedures for extracting the crude carotene and for the chromatographic analysis are briefly summarized as follows:

For fresh green leafy vegetables, pumpkins, and squash five to 10 grams of the edible portion was chopped in 12 per cent alcoholic potassium hydroxide, at room temperature, in a Waring blender for five minutes. For sweet potatoes five to 10 grams were chopped with 95 per cent alcohol as recommended by Lease and Mitchell (1940). For carrots the same

procedure was used except that the alcoholic extract was filtered and then approximately one-half its volume of saturated alcoholic potassium hydroxide was added and the mixture agitated in the Waring blender for two to three minutes. For dehydrated sweet potatoes and carrots one to three grams of the finely ground material was soaked with five to 15 ml. of water until soft, and then treated as above. For canned materials the contents of the can were poured on to a cheesecloth in a funnel and allowed to drain. Then five to 10 grams of the solid material was chopped in the blender. Some of the samples were cooked as specified (Table 1) and then treated in the same way as the fresh samples. The alcoholic mixtures were extracted with petroleum naphtha (Skellysolve F) and the extract purified with methanol at room temperature as in the A.O.A.C. method (1940). The crude carotene extracts were diluted to a suitable volume and an aliquot equivalent to 100 to 200 micrograms of crude carotene was concentrated *in vacuo* to five to 10 ml., transferred to a column of calcium hydroxide, and the zones developed by washing the column with petroleum naphtha. If the zones did not develop readily the column was washed with petroleum naphtha containing one to five per cent of acetone. The zones of impurity A, neo-beta-carotene U, beta-carotene, neo-beta-carotene B, and alpha carotene were separated; the pigments were eluted; and the amounts of each were determined by use of a KWSZ colorimeter. If there was any doubt as to the identity of any pigment, it was identified by means of a mixed chromatogram. In calculating the percentages of each pigment, the total amount of pigment recovered from the column was used as 100 per cent instead of the amount put into the column. This would cause very little error since for all but six samples the recovery of pigments was over 90 per cent.

DISCUSSION OF RESULTS

The results of the analyses (Table 1) show that the crude carotene in raw leafy vegetables averaged six per cent of impurity A, 76 per cent of beta-carotene, and 10 and eight per cent of neo-beta-carotene U and B, respectively. The crude carotene of the cooked leafy vegetables averaged practically the same in percentage of impurity A and neo-beta-carotene U as the raw vegetables but contained less beta-carotene and more neo-beta-carotene B. The crude carotene of the dehydrated leafy vegetables averaged a higher percentage of beta-carotene than that of the cooked leafy vegetables and less neo-beta-carotene B. The crude carotene of the canned leafy vegetables averaged a larger percentage of impurity A and neo-beta-carotene U than that of the fresh, cooked, or dehydrated leafy vegetables and less beta-carotene. The neo-beta-carotene B was less than in the cooked leafy vegetables.

The apricots contained lycopin, which is included in impurity A. The crude carotene of apricots, pumpkin, and squash contains a much higher percentage of impurity A than the crude carotene of the other vegetables. Their content of neo-beta-carotene U is appreciably lower than that of leafy vegetables. The squash and pumpkin also contain alpha carotene. The wide difference in the alpha carotene of four to 28 per cent indicates that the crude carotene of different varieties of pumpkin may contain widely different percentages of alpha carotene.

TABLE 1

Constituents of Crude Carotene of Foods

Food and treatment	Constituents of crude carotene						
	Crude carotene	Impurity A	Neo-β-carotene U	β-carotene	Neo-β-carotene B	Alpha carotene	β-carotene equivalent
Leafy vegetables, raw	*p.p.m.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Beet greens	33	4	10	76	10	0	82
Broccoli, Italian sprouting	12	13	1	80	6	0	83
Collards	26	12	13	62	14	0	69
Collards	54	4	9	78	8	0	83
Collards	59	7	10	77	6	0	80
Mustard greens	41	6	9	78	7	0	81
Spinach mustard	72	6	11	76	7	0	80
Spinach	46	5	12	76	7	0	80
Spinach, Bloomsdale	30	7	7	80	6	0	83
Swiss chard	33	7	13	70	10	0	75
Swiss chard	24	4	16	73	7	0	77
Swiss chard	49	6	9	78	7	0	82
Turnip greens	54	4	9	79	8	0	83
Turnip greens	19	4	10	78	8	0	82
Turnip greens	49	3	10	80	7	0	84
Turnip greens, Shogoin	60	7	10	76	7	0	80
Average (16)	42	6	10	76	8	0	80
Leafy vegetables, cooked							
Beet greens, fresh, boiled 20 min	81	4	13	67	16	0	76
Collards, boiled 20 min	44	9	10	55	26	0	68
Mustard greens, boiled 20 min	56	4	15	65	16	0	73
Mustard greens, boiled 30 min	80	5	20	59	16	0	67
Spinach, boiled 20 min	52	4	12	58	26	0	71
Swiss chard, boiled 20 min	36	8	15	66	11	0	71
Turnip greens, fresh, boiled 30 min	107	5	0	75	17	3	85
Turnip greens, boiled 20 min	66	5	16	62	17	0	70
Average (8)	65	6	13	63	18	0	73
Leafy vegetables, dehydrated							
Beet greens	518	3	12	75	10	0	80
Collards	432	25	11	51	13	0	57
Swiss chard	383	6	10	76	8	0	80
Turnip greens	533	5	13	72	10	0	78
Average (4)	465	10	11	69	10	0	74
Leafy vegetables, canned							
Spinach	54	13	15	61	11	0	70
Spinach	33	30	24	38	8	0	42
Spinach	58	11	17	53	19	0	62
Spinach	70	28	17	45	10	0	50
Turnip greens	40	13	26	51	10	0	56
Turnip greens	74	12	23	49	16	0	57
Average (6)	55	18	20	50	12	0	56
Apricots, canned							
Apricots	17	27	0	69	4	0	71
Apricots	14	31	0	62	7	0	66
Apricots	13	31	6	56	7	0	60
Average (3)	15	30	2	62	6	0	66
Squash and pumpkin, raw							
Squash, Ford Hook	2	32	3	54	7	4	60
Squash, Ford Hook	4	42	4	45	3	6	50
Pumpkin, orange shelled	20	41	2	52	2	3	55
Average (3)	9	38	3	51	4	4	55

TABLE 1 *(Concluded)*

Food and treatment	Constituents of crude carotene						
	Crude carotene	Impurity A	Neo-β-carotene U	β-carotene	Neo-β-carotene B	Alpha carotene	β-carotene equivalent
	p.p.m.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Squash and pumpkin, boiled							
Squash, Ford Hook, boiled 30 min	3	40	7	40	11	3	42
Squash, Ford Hook, boiled 20 min	3	50	6	31	6	8	38
Pumpkin, orange shelled, boiled 30 min	7	49	4	35	8	4	41
Average (3)	4	46	5	36	8	5	40
Pumpkin, canned							
No. 1	38	12	5	47	8	28	65
No. 2	49	18	9	39	9	25	56
No. 3	21	30	10	26	13	21	43
Average (3)	36	20	8	37	10	25	55
Sweet potatoes, raw							
No. 1	50	15	0	77	8	0	82
No. 2	64	7	0	90	3	0	92
No. 3	80	7	0	90	3	0	91
Average (3)	65	10	0	86	4	0	88
Sweet potatoes, cooked							
Baked 70 min	46	17	0	67	16	0	75
Baked 60 min	55	22	0	62	16	0	70
Baked 90 min	43	15	2	68	15	0	75
Baked 90 min	57	13	0	74	13	0	80
Baked 90 min	44	12	0	68	20	0	78
Average (5)	49	16	0	68	16	0	76
Sweet potatoes, dehydrated							
No. 1	131	13	0	76	11	0	81
No. 2	126	13	0	74	13	0	80
No. 3	143	10	6	71	13	0	78
No. 4	134	10	3	72	15	0	79
No. 5	132	13	7	64	16	0	72
No. 6	160	9	0	78	13	0	84
No. 7	156	9	0	81	10	0	86
No. 8	88	17	27	35	21	0	46
Average (8)	116	12	5	69	14	0	76
Carrots, raw							
No. 1	106	3	1	59	4	33	77
No. 2	105	3	2	73	2	20	84
No. 3	133	6	0	52	6	36	73
No. 4	39	7	0	65	0	28	79
Average (4)	96	5	1	62	3	29	78
Carrots, boiled							
Boiled 30 min	121	3	2	50	11	34	72
Boiled 20 min	111	8	0	49	11	32	71
Boiled 30 min	103	2	5	55	15	23	74
Boiled 30 min	118	6	0	44	9	41	69
Average (4)	113	5	2	50	11	32	72
Carrots, dehydrated							
No. 1	1048	3	1	67	3	26	81
No. 2	698	6	0	63	2	29	79
No. 3	750	7	3	63	4	23	77
No. 4	923	18	0	64	5	13	72
No. 5	855	3	0	69	7	21	83
Average (5)	535	7	1	65	5	22	78

The crude carotene of raw sweet potatoes contained a slightly higher percentage of impurity A than that of the fresh leafy vegetables but less neo-beta-carotene B and no neo-beta-carotene U. Cooking the sweet potatoes apparently increased the percentage of neo-beta-carotene B.

The crude carotene of the raw carrots was low in impurity A and in the two neo-beta-carotenes but averaged 30 per cent alpha carotene. Cooking or dehydrating the carrots had little effect on impurity A and neo-beta-carotene U but increased the neo-beta-carotene B.

Cooking the foods, as a rule, decreased the percentage of beta carotene in the crude carotene, increased the percentage of neo-beta-carotene B, and, to a much smaller extent, increased the neo-beta-carotene U. Cooking had little effect on impurity A except perhaps in the sweet potatoes.

As pointed out above, the beta-carotene equivalent is a measure of the amount of vitamin A-active pigments expressed in units of beta-carotene. In decreasing order of percentages the beta-carotene equivalents of the various vegetables are as follows: raw sweet potatoes 88, raw leafy vegetables 80, dehydrated and raw carrots 78, dehydrated and cooked sweet potatoes 76, dehydrated leafy vegetables 74, cooked leafy vegetables 73, cooked carrots 72, canned apricots 65, canned leafy vegetables 56, raw squash and pumpkin 54.7, canned pumpkin 47, and cooked squash and pumpkin 40. It is possible that the canned leafy vegetables used were older than the normal and that the contents of freshly canned material would have higher beta-carotene equivalents.

SUMMARY

The crude carotene extracts of certain vegetables were analyzed for their constituents. Green leafy vegetables averaged six per cent impurity A, 76 per cent beta-carotene, 10 per cent neo-beta-carotene U, and eight per cent neo-beta-carotene B. Raw sweet potatoes averaged 10 per cent impurity A, 86 per cent beta-carotene, 0 per cent neo-beta-carotene U, and four per cent neo-beta-carotene B. Raw carrots averaged five per cent impurity A, 62 per cent beta-carotene, one per cent neo-beta-carotene U, three per cent neo-beta-carotene B, and 29 per cent alpha-carotene. Raw squash and pumpkin averaged 38 per cent impurity A, 51 per cent beta-carotene, three per cent neo-beta-carotene U, four per cent neo-beta-carotene B, and four per cent alpha-carotene. Cooking foods, as a rule, decreased the percentage of beta-carotene and increased the percentage of neo-beta-carotene B. Dehydration apparently did not greatly affect the constituents of the crude carotene.

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EFFECT OF VARIOUS HOME PRACTICES ON ASCORBIC ACID CONTENT OF POTATOES[1]

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This study was undertaken in order to determine how the ascorbic acid content of potatoes was affected by different home procedures. There are numerous reports in the literature concerning the ascorbic acid content of potatoes and several that deal with the effects of advance preparation, of cooking, and of holding the cooked product on the amounts of ascorbic acid present. No attempt has been made here to review all the articles that have been published on these subjects.

Values for the ascorbic acid content of potatoes reported by Bessey (1938b), Harris and Olliver (1942), and Chappell (1940) show that the amount found depends to a great extent upon whether the potatoes under examination have been freshly dug or whether they have been stored. Clagett and Tottingham (1941) studied the ascorbic acid content of different varieties of potatoes; Chippewa potatoes which had been in cold storage for 12 to 14 weeks after harvesting contained from 7.6 to 8.5 milligrams per 100 grams, while Cobbler potatoes under the same storage conditions contained from 9.6 to 13.5 milligrams per 100 grams. Reedman and McHenry (1938) obtained values of 31.4 and 20.7 milligrams per 100 grams for Irish Cobbler potatoes which had been stored six weeks and five months, respectively.

A few studies have been made on the effect of advance preparation on the ascorbic acid content of potatoes. Wachholder, Heidinger, Grieben, and Köhler (1937-1938) reported a loss of 25 per cent in the ascorbic acid content of peeled potatoes which were boiled in salted water and a loss of 26 per cent in one instance when peeled potatoes were allowed to stand for 24 hours in water before boiling. Richardson and Mayfield (1943) found that, while potatoes which had been soaked in salt water for four hours contained more ascorbic acid after boiling and after cooking in a pressure saucepan than those which had not been soaked, potatoes which were allowed to stand in fresh water showed greater losses when cooked. Negligible losses in the ascorbic acid content of potatoes which had been peeled, cut in pieces, and soaked in water for 24 hours, were reported by Lauersen and Orth (1942).

There is a wide range not only in the values reported for the ascorbic acid content of potatoes cooked by different procedures but even in the amounts of change in the ascorbic acid content produced by one cooking method. Results of the effect of baking show the greatest variation. Esselen, Lyons, and Fellers (1942) found an average loss of 49 per cent in the ascorbic acid content when eight potatoes of each of eight varieties

[1] Work undertaken as part of the National Cooperative Experiment Station Project on "Conservation of Nutritive Values of Foods."

were baked. Losses ranging from 19 to 59 per cent were reported by Olliver (1941) while Richardson, Davis, and Mayfield (1937) obtained a gain of 41 per cent and a loss of two per cent for two varieties of potatoes. Rolf (1940) baked Irish Cobbler potatoes to an internal temperature of 96°C.(204.8°F.) and found that the average loss in ascorbic acid content was 15 per cent. Boiling potatoes was observed to cause little change in the ascorbic acid content by Givens and McClugage (1920) and by Thiessen (1936). Woods (1935) noticed little difference in the amounts of ascorbic acid present whether or not the potatoes were peeled before boiling. Boiling was found by Halliday and Noble (1936), Lampitt, Baker, and Parkinson (1943), Levy (1937), and Olliver (1936) to decrease the ascorbic acid content of potatoes. Hoygaard and Rasmussen (1938) found that potatoes cooked in salted water retained more ascorbic acid than those boiled in unsalted water. Scheunert, Reschke, and Kohlemann (1936) found that of the three cooking methods which they investigated the greatest loss occurred when potatoes were boiled without their skins, the least when potatoes were steamed in their jackets, and an intermediate value was indicated when peeled potatoes were steamed. Richardson, Davis, and Mayfield (1937) obtained gains on steaming; Rolf (1940), a 10-per cent loss; and Olliver (1941), losses ranging from 18 to 47 and 23 to 44 per cent for peeled, halved and peeled, uncut potatoes, respectively. Richardson and Mayfield found that the ascorbic acid content of potatoes was decreased 10.9 per cent by cooking in a pressure saucepan. It has been shown by several investigators, among others Janse-Stuart and Bever (1938-1939) and Jenkins (1943), that keeping potatoes hot after cooking results in further losses of ascorbic acid but little work has been done on the effect of holding cooked potatoes in the refrigerator. Esselen, Lyons, and Fellers (1942) found that boiled potatoes stored in the refrigerator for 24 hours lost 74 per cent of their ascorbic acid while without the holding period the loss was 27 per cent.

This work was planned in order to determine the effect of several common procedures on the ascorbic acid content of potatoes. The effect of advance preparation was studied by allowing whole and quartered, peeled potatoes to stand covered with tap water for one- and three-hour periods. Potatoes were cooked by different methods—baking until done and for an additional length of time, boiling peeled and unpeeled, steaming, and cooking in a pressure saucepan—and changes in the ascorbic acid content were determined. The effect of holding peeled, boiled potatoes in the refrigerator for two periods of time was investigated.

EXPERIMENTAL PROCEDURE

Red Triumph and Chippewa potatoes purchased on the open market in January and February, respectively, and Irish Cobbler potatoes grown under the supervision of the Department of Horticulture were used in this study. The Irish Cobbler potatoes were grown on prairie loam soil and were dug on October 26th. Experimental work on Red Triumph potatoes was carried on from January 20th to 29th, on Chippewa potatoes from February fourth to April third, and on Irish Cobbler potatoes from October 26th to November 23rd. All of these potatoes were kept in a small

storage house where the temperature ranged from 1 to 15.6° C. (33.8 to 60°F.). Potatoes were brought to the Home Economics Laboratory on the day they were to be used. In addition, some preliminary work was done on Idaho potatoes which were purchased as needed from March through May.[2]

Ascorbic acid was determined by the titration method of Tillmans, Hirsch, and Hirsch (1932) as modified by Bessey and King (1933) and Bessey (1938a). The raw potatoes were extracted according to the procedure which was used for cabbage by Van Duyne, Chase, and Simpson (1944). When cooked potatoes were extracted, five- to seven-gram samples were weighted into 30-ml. portions of three per cent metaphosphoric acid, re-extracted 10 times with 15-ml. portions of acid, and made up to volume in 200-ml. flasks.

Potatoes were sampled by taking three slices from each potato—one through the center, one halfway between the center and the bud end, and the other halfway between the center and the stem end. Approximately half of each slice was included when raw potatoes were sampled, while amounts ranging from one-fourth to one-half of each slice were used when whole cooked potatoes were sampled. Random sampling was employed when the ascorbic acid content of halved or quartered potatoes was determined.

In order to study the effect of various home procedures on the ascorbic acid content of potatoes, they were prepared in the following ways. The ascorbic acid content of the same number of raw potatoes was determined whenever the ascorbic acid content of potatoes subjected to various treatments was investigated.

Advance Preparation: Red Triumph and Chippewa potatoes of varying sizes were peeled and allowed to stand covered with tap water for one- and three-hour periods at room temperature. Four potatoes were placed in 1,000 ml. of tap water in an enameled bowl at one time and the experiment was repeated three times for each variety of potatoes. The same procedure was carried out with peeled, quartered Chippewa potatoes.

Baking: Chippewa, Irish Cobbler, and Red Triumph potatoes were baked in a preheated gas oven set at 232.2°C. (450°F.) until the thermometer in a potato of similar size to those in the lot being baked for sampling registered an internal temperature of 96°C. (204.8°F.). During the baking period the oven temperature ranged from 198.9 to 221.1°C. (390 to 430°F.). The average lengths of the baking periods were 46, 43, and 41 minutes for the Chippewa, Irish Cobbler, and Red Triumph potatoes, respectively. Five Chippewa or Red Triumph potatoes or nine Irish Cobbler potatoes were baked at one time; four of the last-named variety were removed when an internal temperature of 96°C. (204.8°F.) was attained in the potato holding the thermometer and the remaining four were removed 20 minutes later in order to determine the effect of overbaking on the ascorbic acid content. The Idaho potatoes were baked in a preheated gas oven set at 218.3°C. (425°F.) until the internal temperature of the potatoes was 96°C.

[2] Work on the Idaho potatoes was done by Jessie L. Finley in the authors' laboratory. Separation of extracts containing ascorbic acid from the potato residues was accomplished by filtering with suction. The raw potatoes were extracted eight times and the baked potatoes 12 times.

(204.8°F.). The periods of time required ranged from 30 to 50 minutes. Two potatoes similar in weight and shape were baked at one time; one contained the thermometer and the other was sampled for an ascorbic acid determination. After baking, all of the potatoes were weighed and peeled; ascorbic acid determinations were made on the flesh and in a few cases separately on the baked peelings.

Boiling: Chippewa potatoes were placed in twice their weight of boiling, salted, tap water in an aluminum pan with a fitted cover and cooked to an internal temperature of 96°C.(204.8°F.). The thermometer, to which a 50-gram weight had to be attached to hold it in place, protruded through the steam vent. Five potatoes, either unpeeled or peeled, one carrying the thermometer and the other four being used for analyses, were boiled at one time; both experiments were repeated four times. The average lengths of the boiling periods were 29.6 and 28.6 minutes for the potatoes boiled in their skins and for those which were peeled before boiling, respectively. Details of the cooking procedure—regulation of the gas burners, control of the amounts of water, amounts of salt used, and the draining and weighing of the cooked products—were carried out according to the mimeographed outline of Recommended Laboratory Procedures for Food Preparation and Food Preservation for the National Cooperative Experiment Station Project on "Conservation of the Nutritive Values of Foods."

Steaming: Five peeled Chippewa potatoes, one holding the thermometer, were steamed in an aluminum steamer to an internal temperature of 96°C.(204.8°F.) at one time. This experiment was repeated four times; the average length of time required was 36 minutes. In addition, one lot of unpeeled Chippewa potatoes was steamed for 43 minutes at which time the internal temperature in one of the potatoes was 96°C.(204.8°F.).

Cooking in Pressure Saucepan: Four peeled Chippewa potatoes, which had been cut in halves lengthwise, were cooked for eight minutes at 15 pounds pressure in a two-quart pressure saucepan. Sixty ml. of water were used in cooking. The length of time employed was that judged to bring the potatoes to the same degree of "doneness" as was obtained in cooking to an internal temperature of 96°C.(204.8°F.) by the other methods. The experiment was repeated five times.

Holding Cooked Potatoes: Ten large Chippewa potatoes were peeled, cut in halves, and divided into four lots of approximately the same weight. One lot was sampled immediately for ascorbic acid determinations while the other lots were boiled, by the procedure already described, to an internal temperature of 96°C.(204.8°F.). The average time required was 29 minutes. One lot was sampled after cooking, one after holding in the refrigerator for 24 hours, and the third after holding for 70 hours in the refrigerator. This experiment was repeated four times.

RESULTS AND DISCUSSION

The mean values and the standard deviations of the means for the ascorbic acid content of all of the raw, peeled potatoes studied were 0.088 ± 0.0011, 0.106 ± 0.0034, 0.209 ± 0.0078, and 0.076 ± 0.0023 milligram per gram for the Chippewa, Idaho, Irish Cobbler, and Red Triumph varieties, respectively. The value of 0.088 milligram per gram for Chip-

TABLE 1

Effect of Advance Preparation on Ascorbic Acid Content of Potatoes

Variety of potato	Moisture mean and standard deviation of the mean [1]	Treatment	Number of determinations	Ascorbic acid mean and standard deviation of the mean [1]		Difference between means [2] and standard error of difference [3]
				As determined	Raw-weight basis	
	pct.			*mg./gm.*	*mg./gm.*	
Chippewa	81.4 ± .28	Peeled	12	.092 ± .0019	.092 ± .0019	.....
		Peeled, in water 1 hr.	12	.088 ± .0043	.090 ± .0044	.002 ± .0048
		Peeled, in water 3 hr.	12	.092 ± .0026	.095 ± .0026	.003 ± .0033
Chippewa	82.0 ± .35	Peeled	12	.089 ± .0020	.089 ± .0020	.....
		Peeled, quartered, in water 1 hr.	12	.087 ± .0011	.089 ± .0024	.000 ± .0031
		Peeled, quartered, in water 3 hr.	12	.087 ± .0018	.092 ± .0042	.003 ± .0047
Red Triumph	79.8 ± .27	Peeled	12	.071 ± .0047	.071 ± .0047	.....
		Peeled, in water 1 hr.	12	.073 ± .0029	.075 ± .0030	.004 ± .0056
		Peeled, in water 3 hr.	12	.073 ± .0030	.077 ± .0031	.006 ± .0056

[1] Standard deviation of the mean $= S\bar{x} = \sqrt{\frac{\Sigma x^2}{n(n-1)}}$.

[2] Difference between the means calculated from values of peeled potatoes ($\bar{x}_1$) and from peeled and soaked potatoes ($\bar{x}_2$) on the raw-weight basis.

[3] Standard error of the difference $= \sqrt{(S\bar{x}_1)^2 + (S\bar{x}_2)^2}$.

pewa potatoes which were examined from February fourth to April third, probably about four to six months after digging, is in agreement with the amount (0.085 milligram per gram) found by Esselen, Lyons, and Fellers (1942) for Chippewa potatoes which had been kept in cold storage for five months, but higher than the amount (0.066) found by these workers for potatoes held in common storage. Their conditions of common storage were similar to those under which the potatoes used in this experiment were kept. Likewise the value of 0.209 milligram of ascorbic acid per gram for Irish Cobbler potatoes is higher than that of the above authors for the same variety at the time of digging. The ascorbic acid content as determined in this study, however, falls within the range of figures given by Karikka, Dudgeon, and Hauck (1944) for the ascorbic acid content of Irish Cobbler potatoes tested during one month after harvesting.

Figures for the ascorbic acid content of peeled potatoes, of peeled potatoes that had been allowed to stand in tap water for one- and three-hour periods, and of peeled, quartered potatoes that had been soaked for the same lengths of time are presented (Table 1). The average values obtained show that the ascorbic acid content was not decreased by these treatments while the slightly higher figures for the majority of the soaked potatoes are not significantly different from the untreated ones. Richardson and Mayfield found that cooking after soaking in fresh water resulted in a greater loss than cooking without advance preparation, while after soaking in salt water there were smaller losses than when the potatoes were cooked immediately.

Results obtained by baking four varieties of potatoes to an internal temperature of 96°C.(204.8°F.) and of overbaking one variety for 20 minutes are given (Table 2). It is interesting to note that with the exception of the Red Triumph potatoes the ascorbic acid content of the flesh of the baked potatoes was as high as, in some cases higher than, that of the raw potatoes. However, when the average percentage decreases in weight—16.6, 13.3, 15.5, and 13.5 for the Chippewa, Idaho, Irish Cobbler, and Red Triumph potatoes, respectively, and 26.8 for the overbaked Irish Cobbler potatoes—are taken into account, it is seen that significant losses in the total ascorbic acid content occur. Approximately the same percentage retentions (87, 88, and 88) were obtained when Chippewa, Idaho, and Irish Cobbler potatoes were baked. The per cent retention of ascorbic acid was lower when Irish Cobbler potatoes were overbaked but this value was still higher than that found when Red Triumph potatoes were baked. Esselen, Lyons, and Fellers (1942) reported losses on baking Chippewa and Irish Cobbler potatoes of 45 and 59 per cent, respectively. However, the figures obtained in the present study are very similar to those of Rolf (1940) who baked Irish Cobbler potatoes to an internal temperature of 96°C.(204.8°F.). Extracts of raw and baked skins of Red Triumph potatoes had so much color that the amount of ascorbic acid present could not be determined by the visual titration method. Extracts of the skins of baked Chippewa potatoes reduced approximately five times as much of the 2,6-dichlorophenolindophenol solution as did extracts of the raw peelings. There was evidently some substance other than ascorbic acid in the

TABLE 2

Effect of Baking on Ascorbic Acid Content of Four Varieties of Potatoes

Variety of potato	Moisture mean and standard deviation of the mean	Treatment	Number of determinations	Ascorbic acid mean and standard deviation of the mean		Difference between means [1] and standard error of difference	Retention of ascorbic acid, raw-weight basis
				As determined	Raw-weight basis		
	pct.			*mg./gm.*	*mg./gm.*		*pct.*
Chippewa	79.7 ± .22	Peeled	16	.083 ± .0015	.083 ± .0015	.....	....
		Baked and peeled	16	.087 ± .0021	.072 ± .0019	.011 ± .0024	87
Idaho	.....	Peeled	10	.106 ± .0034	.106 ± .0034	.....	....
		Baked and peeled	11	.107 ± .0029	.093 ± .0026	.013 ± .0043	88
Irish Cobbler	81.0 ± .46	Peeled	16	.209 ± .0078	.209 ± .0078	.....	....
		Baked and peeled	15	.215 ± .0089	.183 ± .0080	.026 ± .0111	88
		Overbaked and peeled	16	.234 ± .0093	.171 ± .0071	.038 ± .0106	82
Red Triumph	79.2 ± .38	Peeled	16	.079 ± .0018	.079 ± .0018	.....	....
		Baked and peeled	16	.073 ± .0024	.062 ± .0016	.017 ± .0024	78

[1] Difference between the means calculated from values of raw and baked potatoes on the raw-weight basis.

baked skins and browned portions of the flesh directly under the skin, which reduced the dye solution.

Figures obtained when Chippewa potatoes were cooked by different methods and when they were stored in the refrigerator for 24 and 70 hours after boiling (Table 3) show that significant losses of ascorbic acid occurred except when potatoes were boiled without peeling. Raw, scraped potatoes contained 0.091 ± 0.0038 milligram of ascorbic acid per gram while boiled potatoes, skinned after cooking, contained 0.094 ± 0.0020 milligram per gram on the raw-weight basis. The difference between these two averages is not considered significant. When potatoes were peeled and boiled or peeled, halved, and boiled, 13 per cent of the original ascorbic acid content was lost. Attempts were made to determine ascorbic acid in the cooking waters; 50-ml. portions of cooking water were made up to volume with six per cent metaphosphoric acid in 100-ml. flasks, and 25-ml. aliquots were titrated with the dye solution. If ascorbic acid was present in these cooking waters, the concentration was too low to be determined by this method. Esselen, Lyons, and Fellers (1942), Halliday and Noble (1936), and Lampitt, Baker, and Parkinson (1943) investigated the effect of boiling on the ascorbic acid content of potatoes and reported higher losses than those obtained in the present study. However, Richardson and Mayfield failed to observe any loss when potatoes were boiled in their jackets and Oser, Melnick, and Oser (1943) reported a loss of 12 per cent when potatoes were boiled by the "old-fashioned" method. In some cases, comparison of the results obtained by different investigators is difficult because the cooking procedures used are only named and not clearly defined. The average ascorbic acid content of peeled Chippewa potatoes was decreased by 14 per cent when they were steamed to an internal temperature of 96°C.(204.8°F.). In one lot of potatoes which were steamed without paring there was no loss of ascorbic acid. Greater losses were obtained by Olliver (1941), who tested potatoes after different periods of storage and found that peeled, halved potatoes lost from 18 to 47 per cent of their ascorbic acid content on steaming. Although the potatoes used in the present study were not halved, when potatoes were boiled, halving them did not affect the amount of ascorbic acid retained. When potatoes were cooked in the pressure saucepan, 85 per cent of the original ascorbic acid content was retained and approximately three per cent was found in the cooking water. The value for the amount lost is slightly higher than the 10.9 per cent loss obtained by Richardson and Mayfield. It is interesting that four methods of cooking Chippewa potatoes should have the same effect on the ascorbic acid content. The per cent retentions obtained—87 after boiling peeled potatoes, 86 after steaming peeled potatoes, 85 after cooking peeled, halved potatoes in the pressure saucepan, and 87 after baking—are without significant difference. The standard deviations of the means for ascorbic acid content, from which the above per cent retentions are calculated, range from ±0.0015 to ±0.0036 and could in each case account for a difference of two per cent in the figures for the percentage retention.

Chippewa potatoes that had been peeled, halved, boiled, and stored in the refrigerator for 24 hours retained 47 per cent of their original ascorbic

TABLE 3

Effect of Different Cooking Procedures and of Holding After Cooking on Ascorbic Acid Content of Chippewa Potatoes

Moisture mean and standard deviation of the mean	Treatment	Number of determinations	Ascorbic acid mean and standard deviation of the mean		Difference between means [1] and standard error of difference	Retention of ascorbic acid, raw-weight basis
			As determined	Raw-weight basis		
pct.			*mg./gm.*	*mg./gm.*		*pct.*
80.4 ± .31	Scraped	16	.091 ± .0038	.091 ± .0038	.....	....
	Boiled and skinned	16	.093 ± .0018	.094 ± .0020	.003 ± .0043	103
80.9 ± .73	Peeled	16	.092 ± .0036	.092 ± .0036	.....	....
	Peeled and boiled	15	.079 ± .0018	.080 ± .0021	.012 ± .0042	87
79.7 ± .29	Peeled	16	.093 ± .0028	.093 ± .0028	.....	....
	Peeled and steamed	16	.081 ± .0022	.080 ± .0016	.013 ± .0032	86
80.0 ± .38	Peeled	20	.089 ± .0020	.089 ± .0020	.....	....
	Peeled, halved, and cooked in a pressure saucepan	20	.077 ± .0022	.076 ± .0031	.013 ± .0037	85
80.2 ± .34	Peeled	16	.076 ± .0025	.076 ± .0025	.....	....
	Peeled, halved, and boiled	16	.065 ± .0019	.066 ± .0028	.010 ± .0031	87
	Peeled, halved, boiled, and stored [2] 24 hr.	16	.038 ± .0031	.036 ± .0050	.040 ± .0056	47
	Peeled, halved, boiled, and stored [2] 72 hr.	16	.014 ± .0015	.014 ± .0012	.062 ± .0028	18

[1] Difference between the means calculated from values of raw and cooked potatoes on the raw-weight basis. [2] In a covered bowl in the refrigerator.

acid content, while after 70 hours of refrigerator storage only 18 per cent was retained. The loss on boiling and holding for 24 hours in the refrigerator is less than that reported by Esselen, Lyons, and Fellers (1942).

SUMMARY

Mean values and standard deviations of the means for the ascorbic acid content of four varieties of peeled potatoes—Chippewa potatoes studied from February fourth to April third, Idaho potatoes from March through May, Irish Cobbler potatoes from October 26th to November 23rd, and Red Triumph potatoes from January 20th to 29th—were 0.088 ± 0.0011, 0.106 ± 0.0034, 0.209 ± 0.0078, and 0.076 ± 0.0023 milligram per gram, respectively.

There were no significant changes in the ascorbic acid contents of peeled Red Triumph potatoes, of peeled Chippewa potatoes, or of peeled, quartered Chippewa potatoes when they were allowed to stand in tap water for one- and three-hour periods.

When potatoes were baked to an internal temperature of 96° C. (204.8°F.), the following percentage retentions of ascorbic acid were obtained: 87, 88, 88, and 78 for Chippewa, Idaho, Irish Cobbler, and Red Triumph potatoes, respectively. Overbaking Irish Cobbler potatoes for 20 minutes caused an additional decrease of six per cent in the ascorbic acid content.

The ascorbic acid content of Chippewa potatoes boiled to an internal temperature of 96°C.(204.8°F.) was not significantly different from that of the raw.

When peeled Chippewa potatoes were cooked to an internal temperature of 96°C.(204.8°F.) by boiling and steaming, the percentage retentions of ascorbic acid were 87 and 86, respectively.

Peeled, halved Chippewa potatoes were cooked in a pressure saucepan to approximately the same stage of "doneness" as was attained in the other methods; 85 per cent of the ascorbic acid content was retained.

When peeled, halved Chippewa potatoes were boiled to an internal temperature of 96°C.(204.8°F.), 13 per cent of the ascorbic acid content was lost. Holding similar lots of potatoes in the refrigerator for 24 and 70 hours resulted in additional losses of 40 and 69 per cent, respectively.

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CAROTENOID CONTENT OF CARROT VARIETIES AND STRAINS[1]

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The carrot root *(Daucus carota, L.)* has been the source of material for much research on carotenoids and it serves as an important commercial source of carotene for experimental and nutritional purposes. Considerable discrepancy has been reported in the literature between the carotenoid content of carrots, as determined by chemical and physical methods and converted to provitamin A activities, and the actual potencies found by animal feeding experiments. This general problem has been reviewed recently by Graves (1942). It appeared possible that in some cases the analytical methods might be unreliable in that nonactive carotenoids were determined and calculated as provitamin A-active pigments. For this reason a spectroscopic study of carrot extracts and fractions was undertaken to provide a basis for more critical evaluation of methods.

Very few carrot varieties and strains have been compared with respect to carotenoid content, and the contents of various parts of the root have not been studied quantitatively and spectroscopically to determine varietal differences. These problems are of considerable practical importance to those interested in the commercial use and value of carrots as a source of provitamin A. This paper reports results obtained in a survey of 16 commercial varieties and 18 special strains.

EXPERIMENTAL PROCEDURE

Extraction and Fractionation Methods: No method tried provided complete removal of the carotenoids in one acetone-hexane extraction (with Waring blender) without preliminary blanching. The pigment remaining in the residue from unblanched carrots was not extracted by hexane or carbon disulfide but usually could be removed completely by a second acetone extraction. Samples of 10 to 15 grams fresh weight were extracted twice in a Waring blender with 125 ml. of acetone. The carotenoids thus removed were transferred to hexane by washing carefully with water three times. Carotenols[2] were removed by washing the hexane solution successively with 90-per cent methanol, 20-per cent methanolic potassium hydroxide, and 90-per cent methanol (25 ml. each). The remaining methanol and alkali were removed with water and the solutions were dried with sodium sulfate, made to 100 ml., and stored at 4°C.(39.2°F.) for spectroscopic analysis.

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[1] Journal Paper No. 164 of the Purdue University Agricultural Experiment Station.

[2] The term "carotenol" is used in this paper to denote the carotenoids extracted from hexane by 90-per cent methanol. The use of this term was discussed by Zscheile (1941). Such extraction should remove dihydroxy compounds but very little of the monohydroxy type, as shown by White and Zscheile (1942).

Sampling: Sampling errors were studied previous to the survey. The distribution of carotenoid pigments within a fresh carrot root (Danvers Half Long) was studied by analyzing cross-sectional slices from tip, center, and top regions for total carotenoids. The tip and center were essentially identical but the top was 50 per cent higher in carotenoid content. Smith and Otis (1941) found greater differences. Within the center slices the outer region of the phloem contained 200 per cent more carotenoids than the inner region and 30 per cent more than the xylem.

One- to two-mm. slices of washed carrot roots (not scraped) were taken from the center where the cross-sectional area was uniform. Slices from two carrots gave reproducible results, but it seemed advisable to take an equal number of slices from each of four carrots to make a 10- to 15-gm. sample.

Spectroscopic Studies: Spectroscopic measurements were made with the photoelectric spectrophotometer used by Zscheile, White, Beadle, and Roach (1942) to determine the standard absorption data of "all-trans"[3] alpha- and beta-carotenes, presented here (Figs. 1 and 2) as characteristic curves for comparison purposes. Absorption values were determined at wave lengths 4305, 4495, 4660, and 4782 Å, which are crossing points of the curves for alpha- and beta-carotenes. Specific absorption coefficients at these wave lengths for hexane solutions are 183, 258, 206, and 228 liters per gram centimeter, respectively. Wave length 4495 Å was chosen as best for superposition of the characteristic curves because it is sufficiently distant from the region of high absorption by zeta-carotene and because curves for carrot extracts agree well at 4660 Å under such circumstances. Absorption at wave length 4782 Å is then a good index of isomerization. For reasons developed below, 4782 and 4900 Å were used for analysis of extracts as two-component systems (alpha- and beta-carotenes). In this paper, ratios of wave lengths refer to ratios of relative absorption value at two indicated wave lengths.

Chromatographic Studies: Calcium hydroxide-super-cel (1:1) columns (1 x 15 cm.) were used to chromatograph certain carrot extracts from the survey. Only those samples were chromatographed which had ratios indicative of pigments other than alpha- and beta-carotenes. Five-per cent acetone in hexane was used to develop these chromatograms; gamma-carotene, lycopene, and zeta-carotene were identified on the column by color, relative position, and rate of movement of the zone during the last stage of development.

Production of Carrots: Sixteen commercial varieties (14 garden varieties plus two Belgian field or stock varieties), 18 special strains, and wild carrots were planted outdoors in April, 1943. They were harvested and analyzed at the age of 130 days, by which time the carotene content should have reached a steady value, according to the work of Barnes (1936). Only typical carrots were studied. There was little variation in the appearance of the special strains, most of which were practically colorless. The more

[3] In this paper, reference to absorption curves or values of pure carotenoids will signify those of the "all-trans" form of the carotenoid unless otherwise noted. For further information on the subject of carotenoid isomerization, consult recent papers by Zechmeister and associates.

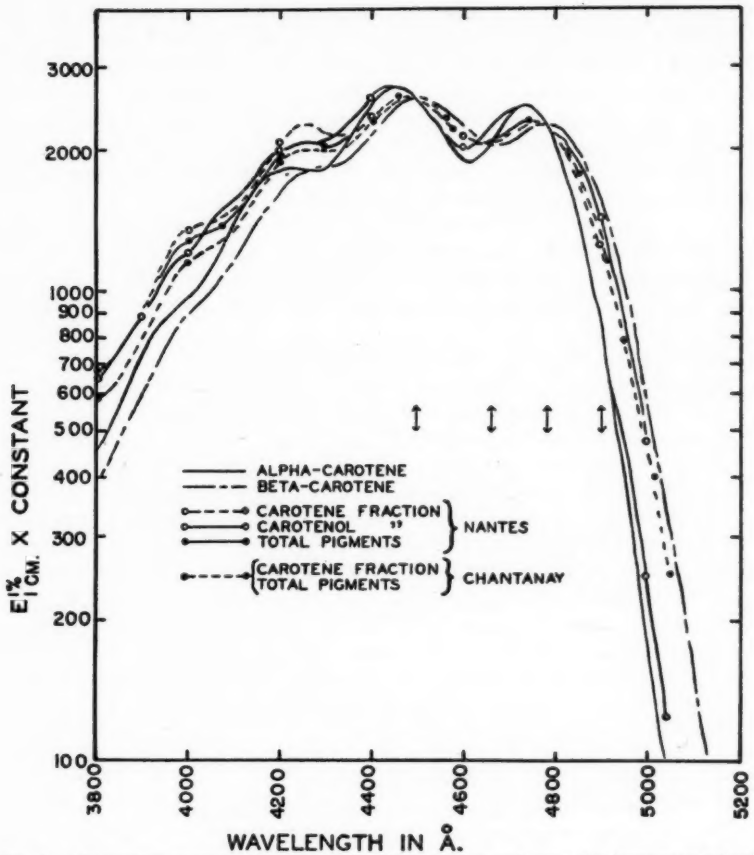

FIG. 1. Absorption spectra of carotenoid extracts of carrot root (in hexane solution).

highly colored special strains were extracted and analyzed (Bureau of Plant Industry Nos. 141959-60 and 142849, -50, -52, -56, -57). Wild carrots were extracted and fractionated but the carotene content was so low that analysis was not practical.

RESULTS AND DISCUSSION

Variety names, colors, and other physical characteristics of the carrots studied in the survey are presented (Table 1). More detailed observations on the carotene fraction are discussed below.

Identification of Carotenoids: The absorption spectra of the total carotenoids and the carotene and carotenol fractions of two typical commercial varieties are presented (Fig. 1). It is well known that the predominant carotenoids of carrot roots are beta- and alpha-carotenes. It was noted earlier by White (1940), and confirmed in the present work, that when the characteristic curves of carrot extracts are superposed to agree at 4495

TABLE 1

Characteristics of Carrot Varieties and Strains

Sample No.	Variety	Color			Length	Overall diameter	Area ratio $\frac{\text{xylem}}{\text{phloem}}$	Total fresh weight	Dry weight
		External	Phloem	Xylem					
					cm.	*cm.*		*gm.*	*pct.*
1	Amsterdam Forcing	Orange	Orange	Orange with red streaks	13	4.2	0.48	120	13.6
2	Belgian Long Yellow	Lemon yellow	Lemon yellow	Yellowish orange	23	3.3	0.81	96	12.7
3	Belgian White	White	White	White	19	2.8	0.85	50	7.35
4	Burpee's Oxheart	Buff orange	Buff orange	Reddish orange	15	5.1	1.10	120	9.45
5	Danvers Half Long	Light orange	Light orange	Light orange	20	3.9	0.80	95	16.2
6	Danvers, Woodruff's Special	Orange	Light orange	Lemon yellow to orange	18	2.9	0.53	72	10.9
7	Early Golden Ball	Light orange	Reddish orange	Light orange	6	3.8	0.50	46	8.7
8	Goldinhart	Orange	Orange	Orange	10	4.1	0.36	74	9.8
9	Hutchinson	Pale orange to yellow	Orange to yellow	Reddish orange to yellow	21	3.0	0.56	69	11.4
10	Imperator	Reddish orange	Dark orange	Dark orange	15	3.1	0.70	68	11.5
11	Improved Long Orange (Co. 1)	Orange	Orange	Orange	18	3.9	1.10	102	12.8
12	Improved Long Orange (Co. 2)	Light orange	Orange	Orange	17	3.2	0.76	63	11.0
13	Morse's Bunching (Co. 1)	Buff orange	Buff orange	Buff orange with red streaks	16	2.6	0.52	57	11.6
14	Morse's Bunching (Co. 2)	Orange	Dark orange	Orange with red streaks	22	4.9	0.94	148	11.0
15	Nantes Half Long	Light orange	Reddish orange	Light reddish orange	14	4.1	0.40	93	11.2
16	Supreme Half Long	Orange	Orange	Orange	14	4.2	0.44	110	11.0
17	Tendersweet	Orange	Orange	Orange with red streaks	20	3.9	0.61	122	11.5
18	Touchon	Light orange	Reddish orange	Reddish orange	13	2.9	0.29	58	9.6
	P. I. numbers[1]								
19	141959	Orange	Orange	Reddish orange	14	4.4	0.54	126	16.7
20	141960	Orange	Orange	Orange and red	10	3.7	0.64	59	13.2
21	142849	Purple	Rich yellow	Purple and yellow	17	2.5	0.44	21	20.8
22	142850	Light red	Light lemon yellow	Whitish yellow	19	3.0	0.34	44	24.2
23	142852	Buff	Lemon yellow	Lemon yellow	23	1.8	0.46	18	17.9
24	142856	Dull orange	Light orange	Orange	11	4.3	0.55	110	9.6
25	142857	Light lemon yellow	Lemon yellow	Whitish lemon yellow	26	3.1	0.43	69	11.6

[1] These P. I. numbers designate strains introduced by the Bureau of Plant Industry, U. S. Dept. Agr.

Å, they agree also at 4660 Å and nearly coincide at 4782Å as well, coincident points of the curves for alpha- and beta-carotenes. Curves of all carrot extracts studied were only slightly lower at 4782 Å than the coincident values of alpha- and beta-carotenes. This indication that the cis-forms of these pigments do not occur to any appreciable extent in fresh carrots was confirmed by the absence of such pigments in the chromatographic studies of the extracts reported (Table 2). This is in agreement with recent work of Kemmerer and Fraps (1943). Many analytical methods employ heat which would promote isomerization and consequent low analytical results.

In earlier work on carrot extracts a red zone had been noticed near the top of magnesia columns. To investigate this zone, carotenes of 50 grams of fresh carrot root (Danvers Half Long) were chromatographed on a magnesia-super-cel (Hyflo) (1:1) column cooled with an ice-water jacket. The colors of the zones from top to bottom were 1) yellowish orange, 2) red, 3) reddish orange, 4) very light yellow, 5) orange, and 6) yellow. Zones 4, 5, and 6 (zeta-, beta-, and alpha-carotenes) were discarded. Zones 1 and 2 (together) and Zone 3 were eluted and rechromatographed as before. No additional zones were found. The yield of pigment from Zone 1 was too low for further work.

Zones 2 and 3 were eluted and the pigments rechromatographed on alumina to give Zones 2a, 2b, and 3a, 3b, respectively. Three per cent acetone in hexane was used to develop both types of columns. The characteristic curves of the pigments were of three distinct types, resembling those of 2a) alpha-, and 3b) gamma-carotenes, and 2b) lycopene. The material from Zone 3a had no definite absorption bands and probably contained oxidized material.

At wave lengths below 4400 Å, the curves for the carotene fractions are usually higher than the standard curves of either alpha- or beta-carotenes, especially near 4000 and 4250 Å. This is due to presence of a carotene, provisionally named zeta-carotene by Strain and Manning (1943), which has major maxima in the same positions as those of the pigment designated as unnamed carotene 1 by White, Zscheile, and Brunson (1942). It was prepared from carrots by them and also by Strain (1939), and Strain and Manning (1943). Absorption at these wave lengths was relatively higher in the carotene fraction from the Nantes carrot, which indicated the carotene nature of this substance. In the cases of Chantanay and Danvers Half Long, the characteristic curves of both the carotene fraction and total carotenoids were identical.

From chromatographic and spectroscopic observations it appears that lycopene was present in the Danvers Half Long carrot studied in a quantity approximating one-tenth that of gamma-carotene. From the position of the pigment having the alpha-configuration on the column (above lycopene), and from its partition between hexane and 90-per cent methanol, it is probable that it was a monohydroxy carotenol (one part to one part gamma-carotene).

Analytical Methods: The possibility of isomerization during the time required in the usual methods of extraction and fractionation was considered. A sample of carrots was extracted in a Waring blender with 125 ml.

TABLE 2

Chromatographic and Detailed Spectroscopic Observations on Carotene Fraction of Carrots

Sample No.	Absorption ratios $\frac{A}{A}$			Additional[2] pigments identified by chromatography	Total carotene content[3]				Composition[4] beta-carotene
					Central cross-sectional basis. γ/g. determined at .....Å				
	$\frac{4960}{4782}$	$\frac{4900}{4782}$	$\frac{4000}{4782}$		4782	4660	4495	4305	(4782-4900 Å)
									pct.
1a[1]	.324	.569	.720	l, z	91.1	95.1	93.1	116.0	62.8
1b	.335	.561	.624	.....	22.1	22.8	22.4	26.2	60.5
2a	.335	.536	1.090	.....	6.58	7.99	7.50	10.7	52.1
2b	.285	.493	.747	.....	5.68	7.00	6.64	8.24	38.4
3a	.410	.607	1.180	.....	0.056	0.066	0.072	0.095	74.9
3b	.442	.623	1.190	.....	0.909	1.01	1.04	1.34	80.1
4a	.306	.525	.782	l, g, z	73.8	67.9	67.6	86.6	48.6
4b	.324	.550	.694	.....	30.4	32.4	33.2	39.7	56.9
5a	.319	.550	.735	l, z	102.0	106.0	104.0	126.0	56.9
5b	.334	.565	.602	l, z	24.0	24.8	23.4	28.0	61.7
6a	.324	.553	.884	z	78.0	81.5	79.6	103.0	57.7
6b	.314	.547	.765	.....	31.7	33.2	32.5	40.0	56.1
7a	.310	.519	.736	l, z	72.7	75.0	71.8	91.4	46.5
7b	.254	.465	.632	no z	21.6	23.6	22.6	26.9	29.6
8a	.325	.554	.697	.....	81.5	83.0	82.9	98.5	57.6
8b	.325	.561	.611	.....	37.8	37.2	36.8	43.9	60.6
9a	.312	.548	.740	.....	38.3	40.0	39.7	48.1	56.1
9b	.292	.486	.682	no z	7.07	7.88	7.37	9.27	36.6
10a	.312	.545	.845	.....	73.8	76.0	66.8	81.2	55.0
10b	.322	.546	.694	.....	21.8	22.7	22.2	26.7	55.2
11a	.301	.526	.732	g, z	91.1	95.5	93.2	114.0	49.2
11b	.298	.521	.625	.....	24.1	25.2	24.5	28.2	61.9
12a	.316	.535	.687	.....	68.8	72.6	71.0	86.2	52.1
12b	.311	.541	.596	.....	30.7	31.9	31.4	34.8	53.7
13a	.356	.....	.834	g, z	68.9	70.9	70.1	89.2	.....
13b	.340	.574	.694	.....	43.0	44.1	43.6	46.5	64.6
14a	.306	.527	.715	.....	44.2	45.2	41.2	54.5	49.2
14b	.308	.516	.565	.....	13.7	14.3	14.0	15.8	46.2
15a	.313	.542	.829	z	70.0	72.5	71.3	89.8	54.4
15b	.298	.520	.672	z	27.8	29.3	27.8	34.3	47.1
16a	.315	.534	.659	l, z	73.1	74.3	74.0	87.4	51.6
16b	.315	.538	.559	.....	48.2	48.3	48.0	54.1	53.1
17a	.358	.....	.692	.....	86.0	87.3	86.3	103.0	.....
17b	.349	.578	.665	.....	32.6	33.3	33.0	39.0	66.0
18a	.347	.567	.922	z	38.4	40.0	38.8	52.0	62.2
18b	.343	.573	.805	z	18.3	19.3	19.0	23.8	64.6
19a	.330	.560	.756	z	64.3	66.8	65.1	80.6	60.4
19b	.325	.548	.733	.....	28.0	29.6	28.7	34.4	56.4
20a	.867	.940	.244	l, g, z	47.4	49.6	48.5	59.5	.....
20b	.856	.898	.650	l, g, z	20.8	21.8	21.6	26.3	.....
21a	.317	.516	.816	.....	2.48	3.46	2.60	3.82	46.1
21b	.318	.505	.890	.....	1.82	2.22	2.28	2.90	41.7
22a	.343	.552	.803	.....	1.16	1.78	1.51	1.81	55.1
22b	.362	.565	1.160	.....	0.40	0.52	0.55	0.72	61.7
23a	.400	.534	.964	.....	0.73	0.94	0.90	1.15	48.6
23b	.531	.616	.890	.....	1.03	1.34	1.22	1.41	78.6
24a	.326	.540	.771	.....	30.90	32.40	31.80	39.20	53.5
24b	.347	.540	.794	.....	9.61	10.70	9.62	12.70	53.5
25a	.349	.488	.946	.....	1.01	1.54	1.32	1.78	31.6
25b	.516	.583	.785	.....	1.58	2.22	1.83	2.34	66.1

[1] "a" numbers represent phloem; "b" numbers represent xylem of the same carrot. [2] l represents lycopene; g represents gamma-carotene; z represents zeta-carotene. In cases where pigments are not noted, the sample was not chromatographed. [3] All analyses are expressed on the fresh-weight basis. [4] The total carotene fraction was analyzed spectroscopically as a mixture of alpha- and beta-carotenes.

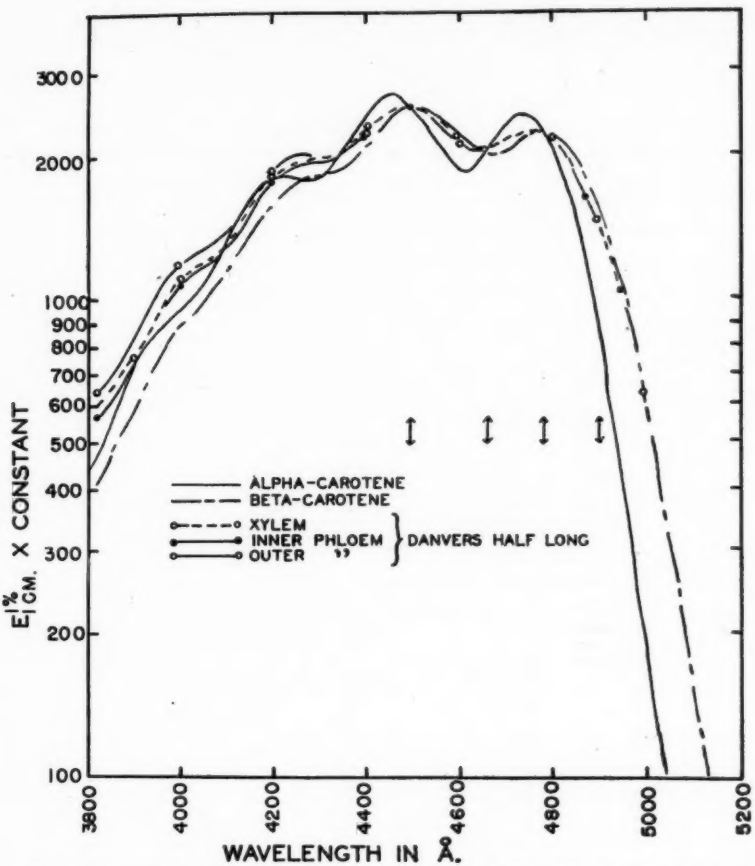

FIG. 2. Absorption spectra of total carotenoids from different sections of carrot root (in hexane solution).

of 50-per cent acetone in hexane. The hexane solution, freed of acetone by washing with water, was dried rapidly by shaking with sodium sulfate. The determination of a spectral absorption curve over the region from 5000 to 3800 Å at 50 Å intervals was started within 30 minutes after the carrot was cut. A second spectrum from 4975 to 3825 Å was started 20 minutes later. These overlapping characteristic curves were identical with each other and with curves of other typical extracts made by the usual longer methods, indicating no difference in isomerization during the different time intervals.

The carotenol fraction of most commercial carrots examined was a relatively small part of the total carotenoids (*ca.* 10 per cent). Danvers Half Long and Yellow Belgian contained larger quantities of carotenols in the xylem (*ca.* 30 to 50 per cent). In the case of White Belgian, this

figure was *ca.* 60 per cent. This fraction often consists in part of the alpha-type of carotenoid (the carotenol fractions of Danvers Half Long and Nantes were predominantly of the alpha type). Cryptoxanthol may be present in the carotene fraction. Any error in calculation of provitamin A content owing to analysis of cryptoxanthol as alpha-carotene would be insignificant because of the similarity in provitamin A potency and the small quantities involved.

The carotenoids from most of the special strains contained from 10 to 50 per cent carotenols in the xylem (considerably more than commercial varieties) and about 10 per cent in the phloem. The carotenoids of wild carrots were almost entirely carotenols (*ca.* 95 per cent) with indication of a high proportion of monohydroxy derivatives.

Although it is usually possible to extract carotenoids completely from fresh carrots with pure acetone after using a mixture of hexane and acetone, complete extraction may be obtained in one step by blanching for four minutes in boiling water before extraction. Mackinney, Aronoff, and Bornstein (1942) employed a three-minute blanch. Their method of extraction was similar to that employed here. Isomerization changes owing to the heat of blanching cause absorption values at 4782 Å to be about three per cent low. Since this error is greater than the usual error, owing to the incompleteness of the second acetone extraction, it appears best to extract fresh unblanched carrots twice, except when unusual cases require more extractions.

The use of wave length 4782 Å appears to be the most reliable as an index of the content of combined alpha- and beta-carotenes. This coincident point was used by Strain (1939) in analysis of butterfat carotenoids (480 mμ in petroleum ether). Wave length 4900 Å is satisfactory for estimation of alpha- and beta-carotene contents individually, although the presence of gamma-carotene or lycopene will cause such results to be high in favor of beta-carotene. Since other pigments of known vitamin A potency occur only to a very small extent, from the practical nutritional viewpoint these determinations are the most significant. White (1939), using 2,2,4-tri-methyl pentane as a solvent, and working on artificial mixtures of pure alpha- and beta-carotenes, demonstrated that wave lengths 4300 and 4670 Å could be used accurately for analysis of total carotene and 4850 and 4950 Å were satisfactory for composition determination. Miller (1934) applied similar methods to such solutions in a solvent composed of 20 per cent ether in ethanol.

Other methods of optical analysis will give various degrees of accuracy. Visual spectrophotometry should give results of practical value for total alpha- and beta-carotenes if wave lengths near 4495 or 4660 Å are chosen. No reliable distinction between these two pigments could be obtained by this method. The use of the 4916 Å line from a mercury arc source appears feasible for distinction between alpha- and beta-carotenes if the total can be reliably determined with some other source of radiation. The 4358 Å mercury line does not occur at a coincident point of the standard curves and is absorbed by other pigments of carrots. With filter photometry, no distinction can be expected between components. Errors in total carotenoid contents might differ among samples if the characteristic curves should

vary considerably in relation to the wave length-sensitivity characteristics of the instrument employed. Factors applicable to the subject of methods were discussed by Zscheile (1941).

Carrot Survey: The root dimensions may be influenced considerably by soil and climatic conditions and the values presented (Table 1) undoubtedly do not represent sizes or relative values attained under other conditions. The soil was heavy and not very fertile. Neither the soil nor the weather conditions were optimal for carrot growth. The area ratios, xylem to phloem, varied over a threefold range; two varieties had approximately equal areas of phloem and xylem but in most cases the phloem exceeded the xylem in area, in agreement with the report of Werner (1941) on relative weights. Xylem-to-phloem ratios varied considerably from tip to top of the carrot. No attempt was made to correct for such variations, because the central region was considered the most representative part of the carrot for practical samples of the size required for routine analysis.

Dry matter content was determined so that calculations of pigment content on the dry basis could be made if desired. A threefold variation was found.

Ratios of absorption values at certain wave lengths, carefully chosen from consideration of the standard curves of pigments known or thought to be present in the extract, are very helpful as an indication of pigment identity. Wide departure of ratios from expected values indicates the presence of additional pigments which may be identified chromatographically or by more complete spectroscopic data (*i.e.* characteristic curves). This general problem was discussed by Mackinney (1935).

The ratios $\frac{4960}{4782}$, $\frac{4900}{4782}$, and $\frac{4000}{4782}$ for mixtures of pure alpha- and beta-carotenes range from 0.167 to 0.434, 0.364 to 0.685, and 0.426 to 0.390, respectively. Among the 50 analyses reported (Table 2), only four ratio values of $\frac{4960}{4782}$ and two of $\frac{4900}{4782}$ were outside the above ranges, all exceptions being on the high side. In Samples 20a and 20b, for which the deviations were greatest, both lycopene and gamma-carotene were identified chromatographically. The presence of these pigments in small quantities in numerous other samples illustrates the fact that an elevation of the ratio by the presence of lycopene or gamma-carotene may be counterbalanced by a suitable change in relationship between alpha- and beta-carotenes. Such ratio ranges considered alone, therefore, are only indications of the identity of pigments but do not exclude the presence of other pigments. The ratio values of $\frac{4000}{4782}$ were all considerably higher than the rather narrow range for alpha- and beta-carotene mixtures, and zeta-carotene was identified chromatographically in all but two of the samples so studied. These data indicate the probable general occurrence of zeta-carotene in considerable concentrations. It has been found in most carrot extracts examined chromatographically in this laboratory. Delta-carotene was not identified. It is probable that additional pigments occur in carrots as minor constituents, although from the practical standpoint

alpha- and beta-carotene are usually the most abundant. Special strain 141960 appears to be an unusual case, with high contents of lycopene and gamma-carotene.

In all cases, the figures for total carotenes calculated from wave length 4305 Å were appreciably higher than those from other coincident points (4495, 4660, and 4782 Å), which indicates further the probable general occurrence of zeta-carotene in carrot roots. Among the garden varieties, agreement between these latter three wave lengths was excellent. Among the special strains, results from 4660 Å were often high and tended to agree with those from 4305 Å. High results at 4660 Å could have been due to the presence of gamma-carotene or lycopene.

The contents of lycopene and gamma-carotene found chromatographically were in no case sufficiently high to cause appreciable errors in the alpha- and beta-carotene analyses. Absorption values of zeta-carotene are negligible at the wave lengths employed (4782 and 4900 Å). The carotene values varied appreciably. Many varieties contained approximately equal percentages of beta-carotene in the phloem and xylem portions of the root but in a few varieties the differences were considerable.

Under total carotenes (Table 3), contents are expressed as micrograms per centimeter (linear with the root axis) of carrot tissue. From these data and dimensional measurements the content (Column 2) was calculated as micrograms per centimeter of carrot length, including phloem and xylem in the proportions found in the central slice. Finally, the total carotene content is expressed on the basis of the whole carrot slice, as micrograms per gram of fresh weight (assuming uniform specific gravity of one). From the practical viewpoint, the last calculation is the most significant.

With the exception of White Belgian, the phloem contained more carotene than the xylem per unit length of root, in agreement with the work of Werner (1941).

The Belgian varieties were very low in total carotenes. Garden varieties, varying over a twofold range, averaged 54 micrograms per gram of fresh carrot. In the previous season of 1942, Nantes and Danvers Half Long carrots contained 52 and 77 micrograms of total carotenes per gram, respectively. Chantanay carrots from the 1942 crop contained 108 micrograms per gram. This figure may be higher because these carrots were grown in northern Indiana under more favorable conditions. No correlation of carotene content with dry weight was found. Frequently the outside appearance of the carrot is a rough index of carotene content but this is not always true. The two Danvers varieties contained equivalent amounts of carotenes, as did the two lots of Improved Long Orange from different sources, but Morse's Bunching from two sources showed wide differences. The special strains varied widely in contrast to the comparatively narrow range of the garden varieties. This probably results from a genetic background that is common to the well-established garden varieties.

Bills and McDonald (1932) studied 10 carrot varieties and insofar as comparison may be made, most of their results were higher than those of Table 3. Inclusion of carotenols in their analysis might explain part

of these differences. Werner (1941) reported carotene contents of Chantanay and Nantes carrots considerably higher than those reported here, but he did not describe the analytical methods used. Smith and Otis (1941) and Mackinney, Aronoff, and Bornstein (1942) found considerably higher carotene contents in carrots than those presented (Table 3) but did not report the varieties studied. Environmental conditions peculiar to the season and soil may have been responsible for the relatively low carotene contents in Table 3, with respect to those found by other workers and in this laboratory in other years. Intervarietal comparisons made here appear valid unless great differences exist between strains in their response to environmental changes.

Scheunert and Wagner (1940) reported contents of beta-carotene approximately equal to those for total carotenes in the garden varieties of Table 3. Considerable discrepancy is thus apparent when the high content of alpha-carotene is considered. It is not clear why these workers apparently ignored the alpha-carotene content of carrots.

General Considerations: Smith and Otis (1941) and Graves (1942) have discussed various reasons for many disagreements between chemical or physical methods of carotene determinations and biological assays on carrots for provitamin A activity. Graves emphasized the necessity for discounting the physicochemical determinations of carotene content by a factor as high as six when estimating the provitamin A content, and reviewed numerous other contributions. On the other hand, Barnes (1936) reported higher biological values for carrots than could be accounted for by the carotene content. He used a colorimetric method with a potassium dichromate standard and did not remove carotenols. Hauge, Westfall, Wilbur, and Hilton (1944) found that two groups of cows produced butters of equal vitamin A potencies when fed equilibrated amounts of carotene in carrot root oil (approximately 25 per cent alpha- and 75 per cent beta-carotenes) and in dehydrated alfalfa hay. Mackinney, Aronoff, and Bornstein (1942) have discussed various problems relating carotenoid analysis to nutritional needs.

The percentages of beta-carotene for 14 garden varieties were weighted according to phloem and xylem carotene content (at 4782 Å) and averaged to obtain a value of *ca.* 54 per cent, which is considerably lower than is often assumed for carrots. Recently Kemmerer and Fraps (1943) reported values as low as 65 per cent and accounted for 94 per cent of the total carotenoids as alpha- and beta-carotenes separated chromatographically. The average percentage of alpha-carotene reported here (46 per cent) is higher than the highest value (35 per cent) found by Mackinney (1935) in a survey of leaf carotenes from 59 species of plants. It is also much higher than the value of five to 15 per cent which Mackinney, Aronoff, and Bornstein (1942) rightfully considered of little consequence from the nutritional viewpoint.

Results of the present investigation do not provide a basis for further discussion of the differences between biological and analytical results referred to above. However, the high percentages of alpha-carotene found in the carrots studied would be an important factor in the estimation of provitamin A activity. If this composition is of general occurrence in

TABLE 3

Carotene Content of Carrot Varieties and Strains

Sample No.	Total carotene content[1]		
	Root basis (calculated)		
	γ/cm. of carrot length	γ/cm. of carrot length (whole)	γ/g. of carrot (whole)
1a[2]	850		
1b	100	950	68
2a	31		
2b	22	53	6.2
3a	0.18		
3b	2.6	2.8	0.45
4a	710		
4b	330	1040	52
5a	570		
5b	130	700	58
6a	340		
6b	72	410	62
7a	550		
7b	80	630	57
8a	790		
8b	130*	920	71
9a	170		
9b	18	190	27
10a	320		
10b	68	390	51
11a	520		
11b	150	670	56
12a	320		
12b	110	430	53
13a	240		
13b	42	280	53
14a	430		
14b	120	550	29
15a	660		
15b	110	770	59
16a	700		
16b	200	900	64
17a	630		
17b	150	780	65
18a	200		
18b	28	230	35
19a	640		
19b	150	790	53
20a	310		
20b	90	400	36
21a	8.4		
21b	2.8	11	2.2
22a	6.1		
22b	0.7	6.8	0.97
23a	1.2		
23b	0.79	2.0	0.8
24a	290.0		
24b	50.0	340.0	23.0
25a	5.4		
25b	3.6	9.0	1.2

[1] See footnote 3, Table 2. [2] See footnote 1, Table 2.

carrots, it should not be overlooked in such comparisons because it might easily cause errors of 25 per cent in estimations of provitamin A content.

SUMMARY

The carotenoid system of carrots was studied spectroscopically and chromatographically. A spectroscopic analytical method was applied to a survey of 16 commercial varieties and 18 imported strains.

Zeta-carotene appears to occur in considerable quantities rather generally in carrots, and lycopene was identified in numerous varieties. The content of cis-isomers of alpha- and beta-carotenes is very low in fresh carrots. Heat applied during analytical procedures may cause error owing to isomerization.

Garden varieties average 54 micrograms per gram total carotenes, varying over a range of about twofold. The percentage of alpha-carotene in these strains averaged 46 per cent, making it very significant in the estimation of provitamin A contents.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the assistance of numerous individuals in the progress of these studies. The early indications of zeta-carotene were noted by J. W. White, Jr. Data of Fig. 1 for the Nantes carrot were supplied by H. A. Nash. Rex Thomas supplied seed for the special strains collected and introduced by the Bureau of Plant Industry and supplied to Purdue through the courtesy of W. E. Whitehouse. Various members of the Department of Botany and Plant Pathology assisted in the production of the carrots. Chantanay carrots were supplied by the Horticulture Department.

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REACTION RATES FOR DECOMPOSITION OF THIAMIN IN PORK AT VARIOUS COOKING TEMPERATURES

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Although the thermolabile properties of thiamin were recognized in early investigations of the vitamin B-complex, there is little definite information regarding the mechanism of loss of this vitamin during the cooking of natural products. Factors other than heat appear to be involved since various foodstuffs show different rates of thiamin loss under identical temperature conditions. A more complete understanding of the nature of thiamin decomposition during heating may lead to the development of cooking procedures which will permit greater retention of thiamin in the food.

Since pure thiamin is cleaved into the thiazole and the pyrimidine constituents when heated in neutral solutions, Williams (1938), it has been commonly assumed that this cleavage is involved in the thermal destruction of thiamin in foods. However, thiamin in natural products is notably more resistant to heat than pure thiamin in aqueous solutions—Melnick, Robinson, and Field (1941) and Greenwood, Beadle, and Kraybill (1943) —thus indicating the existence of factors which can modify the reaction. Greenwood *et al.* showed that cocarboxylase is only slightly more resistant to moist heat than thiamin itself. Hence, it does not seem probable that the natural occurrence of thiamin as part of the cocarboxylase molecule is sufficient to explain its superior retention in foods as compared with aqueous solutions.

Beadle, Greenwood, and Kraybill (1943) confirmed earlier work showing thiamin to be much more stable in solutions of low pH than in neutral or alkaline solutions, and also pointed out the importance of the buffer systems involved. According to these workers, retentions vary from 0 to 97 per cent in different solutions of the same pH, depending upon the buffer used. On the other hand, storage for a week at 49°C.(120.2°F.) has been shown to cause thiamin losses ranging from 10 to 80 per cent in food mixtures of approximately the same pH and having to a large extent the same buffer systems—Rice, Beuk, and Robinson (1943).

Sealock, Livermore, and Evans (1943) have demonstrated the presence of a thiamin-destroying enzyme in fresh fish tissue. This enzyme, however, is heat labile and, hence, its presence could not explain differences in the rate of loss of thiamin during the cooking of foods. Moreover, natural products ordinarily lose thiamin more slowly than pure solutions, but the enzyme accelerates the breakdown of thiamin in fish.

Many investigators have observed that the rate of loss of thiamin increases as the temperature is raised and that the amount lost increases with the duration of cooking. Whether or not such increases are strictly proportional to time and temperature has not been critically investigated heretofore, although several workers have stated that the losses are greatest

during the early stages of cooking—Greenwood *et al.* (1943) and Knott (1942). Watanabe (1939) showed the thermal destruction of pure thiamin in aqueous solutions to proceed according to a first-order reaction but did not determine whether this holds for food materials. During the preparation of this paper Greenwood, Feaster, Jackson, and Kraybill (1944) reported the effects of temperatures of 98.9 to 121.1°C.(210 to 250°F.) upon the thiamin in pork luncheon meat and observed that their data could be interpreted on the basis of a first-order reaction. Their work is complemented by the present investigation which is largely concerned with reaction rates at temperatures below 210°F.

EXPERIMENTAL PROCEDURE

To determine the rates at which thiamin is lost from lean pork muscle during heating, a series of one-half-inch, screw-cap tubes, each containing a five-gram portion of well-mixed, ground, lean pork trimmings was prepared for temperature studies at 49, 54, 63, 71, 77, 82, 93, 99, and 121° C. (120.2, 129.2, 145.4, 159.8, 170.6, 179.6, 199.4, 210.2, and 249.8° F.). All tubes were closed with aluminum caps and sealed with gilsonite (a high-melting wax) to prevent any loss of moisture during the heat treatments. These were conducted in a thermostatically controlled water-bath constant to within ± 0.5°C. Except for the 99°C. and 121°C. series, the samples were preheated to the experimental temperature by immersion in a large volume of water which was five to 10 degrees warmer than the desired sample temperature. When a thermometer inserted in an extra sample indicated that the meat had reached the proper temperature, the tubes were transferred to the water bath and held there for the various intervals of time. These preliminary warmings never required more than four minutes, and analyses indicated negligible destruction of thiamin within that time. Tubes for the 99°C. run were not preheated but were placed directly in boiling water. In all cases intervals were measured from the time the samples reached the cooking temperature. Since it was impossible to apply this technique to the 121°C. experiment, a pair of control samples was placed in a household pressure cooker containing a small quantity of boiling water. The cover was quickly fastened into place, the escape jet remaining open until live steam began to emerge. In approximately two minutes a 15-pound pressure was attained. This pressure was maintained for an additional two minutes, after which the pressure cooker was plunged into cold water and opened for removal of the samples. With a little practice two operators were able to carry out these manipulations in a total of five and one-half minutes. Each set of samples at the 121°C. temperature was held in the cooker for two minutes longer than the indicated period. Losses for the different time intervals at 121°C. were corrected by subtracting the amount lost by the control, this being 4.5 per cent. This procedure accounted for the effects of the warming and cooling operations.

The tubes were chilled immediately after removal from the cooking bath and then held in a cooler at —29°C.(—20.2°F.) until the entire set had been collected. The contents of the tubes were then thawed, crushed, and washed into 100-ml. volumetric flasks with 50-ml. portions of hot N/7.5

H_2SO_4. After extraction in a boiling-water bath for one hour, the samples were treated with takadiastase suspended in a buffer solution, Conner and Straub (1941), diluted to 100 ml., and filtered. Thiochrome analyses of the extracts were made directly, since blanks on the extracts were only slightly higher than on eluates from Decalso adsorption columns, Hennessy and Cerecedo (1939). Variations between duplicate samples rarely exceeded three per cent. Each of the values in the tables represents the average of duplicate analyses checking within ± five per cent.

TABLE 1

Retention of Thiamin in Pork After Heating for Various Intervals and Temperatures

Time	Thiamin retained at									
	49°C.	54°C.	63°C.	71°C.	77°C.	82°C.	88°C.	93°C.	99°C.	121°C.
hr.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
0	100[1]	100[1]	100[2]	100[2]	100[2]	100	100	100	100	100
½	.....	.....	.....	.....	.....	95	.....	.....	83	52
1	.....	.....	.....	.....	.....	91	86	85	75	26
2	.....	.....	.....	93	92	85	73	69	56	9
4	.....	.....	89	85	84	72	58	50	32	.....
8	.....	89	80	71	72	57	38	31	.....	.....
16	.....	84	64	52	48	32	15	10	.....	.....
24	79	73	57	44	34	.....	.....	.....	.....	.....
48	.....	62	36	26	19	10	.....	.....	.....	.....
72	71	51	30	16	.....	.....	.....	.....	.....	.....
96	.....	45	22	.....	.....	.....	.....	.....	.....	.....
120	.....	40	.....	.....	.....	.....	.....	.....	.....	.....
144	.....	37	.....	.....	.....	.....	.....	.....	.....	.....
168	56	32	.....	.....	.....	.....	.....	.....	.....	.....

[1] Same mixture of pork used in 49 and 54°C. runs. [2] Same mixture of pork used in 63, 71, and 77°C. runs.

The data obtained showing the general effects of time and temperature on the loss of thiamin are presented (Table 1 and Fig. 1). Although the data in this form are difficult to interpret quantitatively, it is obvious that the loss of thiamin is proportional to the reaction temperature and its duration. However, when logarithms of the percentages of retention are plotted against time, the resulting curves are straight lines for all temperature series above 77°C. (Fig. 2a), in each of which cases the rate of loss seems to be constant and, therefore, indicates a monomolecular type of reaction. Below 77°C., however, there is a tendency for the reaction rates to become slower with increasing time (Fig. 2b).

That the slowing of reaction rate is not an illusion created by errors in drawing the curves may be proved by mathematical treatment of the data. If the reaction involved in thiamin loss is monomolecular, then reaction constants calculated from the equation $k \frac{2.303}{t_2-t_1} \log \frac{C_1}{C_2}$[1] should be alike for different portions of any one curve. Allowing for experimental

[1] k = reaction constant, t_1 and t_2 = time in minutes at the beginning and end of the interval for which k is being determined, and C_1 and C_2 = concentrations (or per cent retentions) at t_1 and t_2.

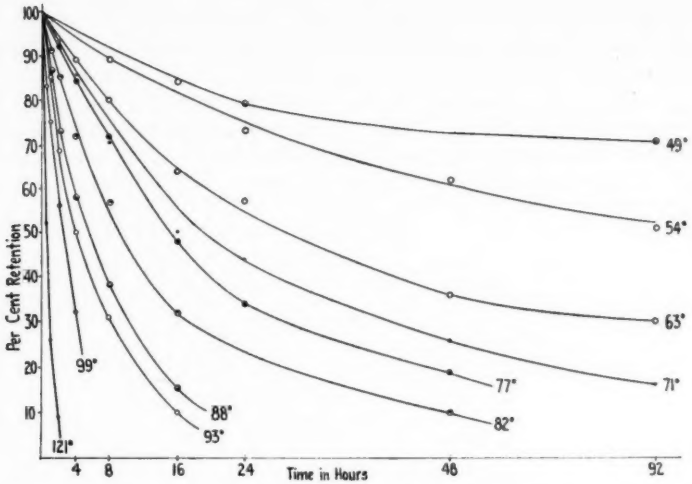

FIG. 1. Per cent retention of thiamin during heating at various temperatures.

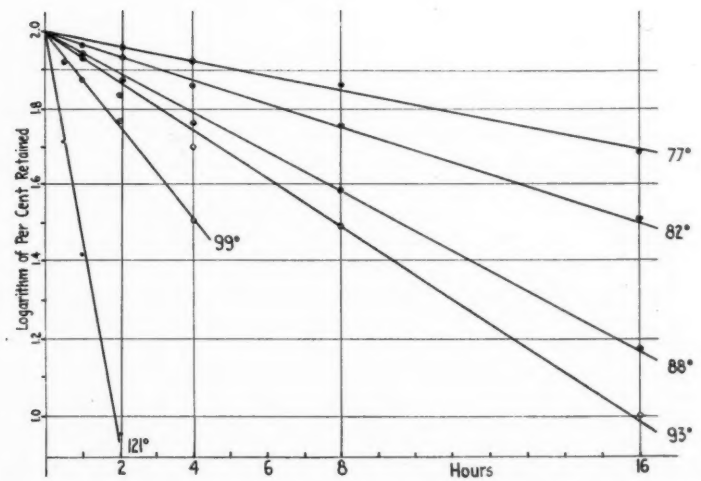

FIG. 2a. Logarithms of per cent retention of thiamin during heating at various temperatures.

variation, this proves to be the case for data representing the series above 77°C. (Table 2). The k values for each of the series show variations throughout, having a definite tendency to become progressively smaller at 71°C. In fact, the reaction constants for each of three lower temperatures appear to be divided into two groups, one for samples taken at or before 16 to 24 hours, and the other for the samples held for longer periods.

TABLE 2

Reaction Constants for Loss of Thiamin in Pork Held at Various Temperatures

Time	Reaction constants × 10^6									
	49°C.	54°C.	63°C.	71°C.	77°C.	82°C.	88°C.	93°C.	99°C.	121°C.
hr.										
0-½	.....	.....	.....	.....	.....	.....	.....	.....	.....	360
½-1	.....	.....	.....	.....	.....	.....	.....	.....	.....	380
0-1	.....	.....	.....	.....	.....	26	42	46	78	.....
1-2	.....	.....	.....	.....	.....	19	45	56	81	340
0-2	.....	.....	.....	10	12	.....	.....	.....	.....	.....
2-4	.....	.....	.....	12	16	23	32	45	80	.....
0-4	.....	.....	8	.....	.....	.....	.....	.....	.....	.....
4-8	.....	.....	8	12	11	16	29	32	.....	.....
0-8	.....	4	.....	.....	.....	.....	.....	.....	.....	.....
8-16	.....	2	8	11	14	20	32	39	.....	.....
16-24	.....	5	4	6	12	.....	.....	.....	.....	.....
24-48	.....	2	5	6	10	.....	.....	.....	.....	.....
48-72	.....	2	2	5	.....	.....	.....	.....	.....	.....
72-96	.....	2	4	.....	.....	.....	.....	.....	.....	.....
96-120	.....	1	.....	.....	.....	.....	.....	.....	.....	.....
120-144	.....	1	.....	.....	.....	.....	.....	.....	.....	.....
144-168	.....	2	.....	.....	.....	.....	.....	.....	.....	.....
0-24	3	.....	.....	.....	.....	.....	.....	.....	.....	.....
24-72	1	.....	.....	.....	.....	.....	.....	.....	.....	.....
72-168	1	.....	.....	.....	.....	.....	.....	.....	.....	.....
Av.....	(3-1)[1]	(4-2)[1]	(8-4)[1]	(11-6)[1]	13	21	36	44	80	340
$t_{½}$,[2](hr.).....	200[3]	72[3]	30	17	16	9	5.4	4.4	2.4	0.6

[1] Average represents the first and second "apparent" reaction constants.

[2] Calculated from the equation $t_{½} = \frac{.693}{K}$.

[3] Estimated from a curve representing the analytical data.

Since the reaction constants are based on differences in retention at two time intervals, it is obvious that the k values will show greater variation than the actual analytical deviations. An error in the analysis of sample taken at any time, say t_x, will influence two k values—the one for the period ending at t_x and also the one for the period starting with t_x—one of the k values being too high and the other too low. As Roseveare (1931) has pointed out, these errors cancel in averaging a series, and the average k is equal to that for the interval between the first and last points.

Since several different mixtures of ground raw pork were used for the samples in the temperature series represented (Tables 1 and 2), the k values cannot be compared directly without introducing complicating factors, such as the pH of the meat, percentage of fat present, etc. At relatively high temperatures these factors will not greatly alter the reaction

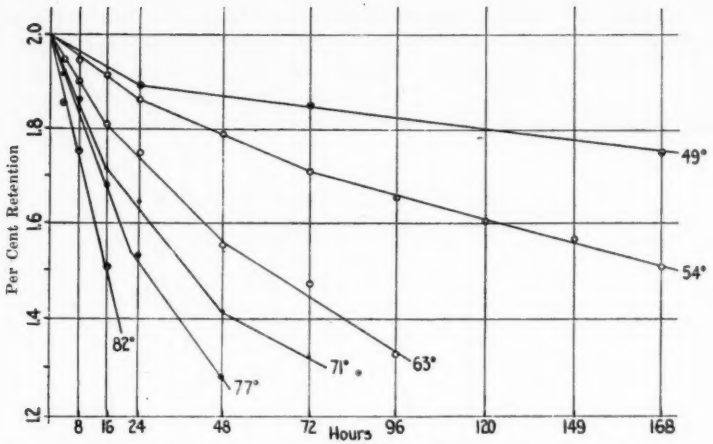

FIG. 2b. Logarithms of per cent retention of thiamin during heating at various temperatures. The scale has been altered from that of Fig. 2a to permit inclusion of longer time intervals.

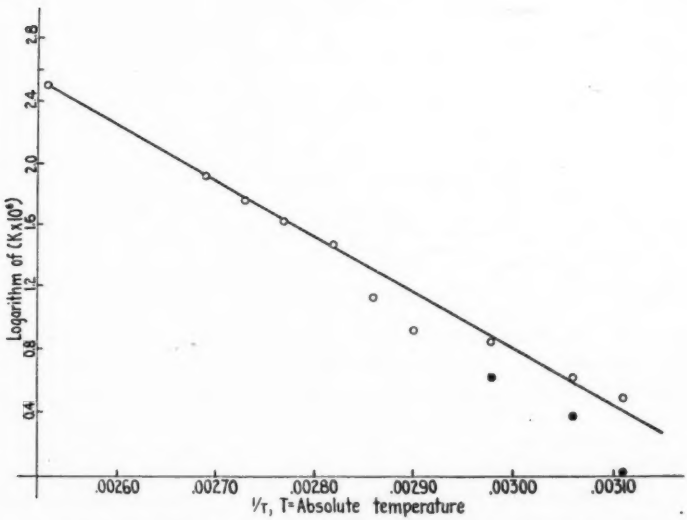

FIG. 3. Relationships of reaction constants to temperatures. Circles indicate constants for several samples of a single mixture of pork for time intervals up to 24 hours. Solid dots indicate reaction constants for duplicate samples but for periods above 24 hours.

rate, as is shown from the fact that four different samples with pH values of 5.5, 5.7, 5.9, and 6.0 showed 50, 53, 51, and 54 per cent retentions, respectively, after four hours at 93°C. At lower temperatures, differences in the meat appear to affect the retention of thiamin to a greater extent, retention for three different samples held at 63°C. for eight hours being 75, 89, and 84 per cent.

Therefore, another series of tubes was prepared from a single mixture of ground pork in order to determine the relationship of the rates of loss to temperature. Duplicate tubes from this series were then held at the various temperatures for one, three, or eight hours, as shown (Table 3). The k values calculated from the analyses obtained in these samples may be compared because of the homogeneous nature of the original samples. Above 77°C. the values for log $(k \times 10^6)$ lie on a straight line when plotted against $\frac{1}{T}$, thus satisfying the conditions of the Arrhenius equation and proving the constants to be directly proportional to the reaction temperature (Fig. 3).

TABLE 3

Reaction Rates for Loss of Thiamin in a Single Mixture of Pork at Various Temperatures

Heating period	Temperature	Retention	Reaction constant	Half-life [1]
hr.	*°C.*	*pct.*	*$\times 10^6$*	*hr.*
1	121	33	320	.6
3	98	41	81	2.4
3	93	54	57	3.4
3	88	64	42	4.6
3	82	72	30	6.4
3	77	87	13	16.0
3	71	92	8	24.0
8	63	81	7	27.4
8	54	89	4	48[2]
24	49	79	3	64[3]

[1] Calculated from the equation $t\frac{1}{2} = \frac{.693}{K}$, where K = reaction constant.

[2] Half-life experimentally obtained by holding sample until only 50 per cent remained, 75 hours.

[3] Half-life experimentally obtained by holding sample until only 50 per cent remained, 200 hours.

At 77°C. and below, however, the points deviate from the line established by the reaction constants of the higher temperatures. The meat used in the series at 54 and 49°C. was part of the same mixture used in obtaining the data in Table 2; and the reaction constants for these temperatures as shown (Table 3) are taken from the respective eight- and 24-hour samples listed in Table 2. Therefore, each of the values for the reaction constant obtained at each of these temperatures (Table 2) may be plotted. When both are used, the lower values deviate markedly from the straight line, whereas the high values, i.e., those representing the early stages of the reaction, fall more nearly on the previous curve (Fig. 3). This change in rate after 16 to 24 hours has been observed in each of 12 different series at 71°C. or below. Pork which had been autoclaved at 13 pounds for seven minutes showed the same percentage loss on subsequent

storage at 54°C. as did raw pork or pork containing curing ingredients commonly used in pork luncheon meats. Likewise, at 49°C., the inclusion of a mixture of cresols to inhibit any possible bacterial effect did not result in a rate of loss different from that in an untreated set of duplicates.

DISCUSSION

At relatively high temperatures (above 77°C.) the loss of thiamin from pork proceeds at a constant rate throughout the entire heating interval. The reaction at these temperatures, therefore, appears to be monomolecular and is probably hydrolytic in nature.

At temperatures below 77°C., however, some change in the reaction occurs during heat treatment, since the reaction rate is much greater during the first 16 to 24 hours than in later intervals. The apparent constancy of the rate after the initial change makes it seem possible that the reaction during the latter stages is monomolecular. However, if this is the case, the low-temperature monomolecular reaction either must be different in nature from that at higher temperatures or must be influenced by some catalytic agent whose effectiveness changes at approximately 77°C., since logarithms of these constants do not fall on the curve established by constants for higher temperatures.

The temperature of the transition point makes enzymatic activity appear to be a logical basis for explaining the change in rate, especially since an enzyme which destroys thiamin has been isolated, Knott (1942). On the contrary, the fact that raw and autoclaved (121°C., seven minutes) pork show similar rates of loss makes this improbable. Moreover, at low temperatures the reaction rates are below those indicated by extrapolation of the Log k *versus* temperature of reaction curve for high temperatures. In order that this phenomenon might be explained by enzyme activity, it would be necessary to postulate a resynthesis of thiamin or a repression of the reaction involved in its loss.

While such studies of the kinetics of the thermal decomposition of thiamin do not permit definite conclusions regarding the nature of the mechanism, they do indicate a slowing of the reaction at 71 to 77°C. This decreased rate of loss may explain the excellent retention of thiamin during the smoking of hams, which involves holding them for long intervals at temperatures below 63°C., as compared with much greater losses for shorter periods of roasting at only slightly higher temperatures, Schweigert, McIntire, and Elvehjem (1943). It likewise indicates that the holding of foods at steam-table temperatures may not be so destructive of this vitamin as has been commonly supposed.

It is hoped that future studies of the factors involved in thiamin decomposition will lead to information by which processing operations can be directed toward minimum vitamin losses.

SUMMARY

The chemical kinetics of the thermal destruction of the thiamin in pork has been investigated at a number of temperatures.

At temperatures above 77°C., the rate of loss of thiamin is constant at any given temperature and is proportional to the temperature.

Below 77°C., the rate of loss decreases during the first 16 to 24 hours, and then apparently remains constant thereafter. In this lower temperature range, the rates of loss are proportional to the temperature.

Half-life periods for thiamin in pork heated at constant temperatures of 49, 54, 63, 71, 77, 82, 88, 93, 99, and 121°C. are, respectively, 200, 75, 27.4, 24, 16, 6.4, 4.6, 3.4, 2.4, and 0.6 hours.

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GROWTH OF *STAPHYLOCOCCUS AUREUS*, *SALMONELLA ENTERITIDIS*, AND ALPHA-TYPE STREPTOCOCCUS EXPERIMENTALLY INOCULATED INTO CANNED MEAT PRODUCTS[1]

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Staphylococcus aureus and *Salmonella enteritidis* are among the bacteria commonly reported as etiological agents of outbreaks of food poisoning, and less commonly alpha-type streptococci are responsible for cases of acute gastroenteritis. Canned foods, as a class, are not associated with outbreaks due to these organisms, although conceivably the food may become contaminated with them after the can is opened. A rare exception to this statement has been previously reported by Cary, Dack, and Meyers (1931). Growth of organisms experimentally inoculated into certain types of canned foods is more luxuriant in some than in others (Segalove, Davison, and Dack, 1943; Surgalla, Segalove, and Dack, 1944; and Segalove and Dack, 1944), and at times growth occurs without elaboration of toxin (Davison and Dack, 1942).

The purpose of the present investigation was to study (1) growth and survival of food-poisoning strains of *Staph. aureus*, *S. enteritidis* and alpha-type streptococci in experimentally inoculated corned beef, roast beef, and potted meat, and (2) the spread of one of these bacteria, *Staph. aureus*, from a single point of inoculation through the contents of the can.

EXPERIMENTAL PROCEDURE

Food-poisoning strains of organisms, *Staph. aureus* No. 161, *S. enteritidis* and alpha-type *Streptococcus* No. 1 were used throughout these experiments.

Canned roast beef, corned beef and potted meat, and corned beef and roast beef that had been specially ground before processing were utilized. The specially ground products and untreated potted meat (which was ground) were studied in the experiments on growth and survival of organisms, and both untreated and special preparations were used in the determination of spread of bacteria through the contents of the can.

In the experiments on growth and survival of organisms in meat products the following technique was employed: cans of the meat products were opened aseptically in a dust-proof cabinet, the contents placed in a sterile mortar and inoculated with one ml. of a diluted, 24-hour broth culture of the organisms. After trituration, one- to two-gram portions of inoculated meat were transferred to small sterile tubes with cotton plugs. A sterile alemite gun was utilized in inoculation of the tubes (technique

[1]This work was aided by a grant from the National Canners Association.

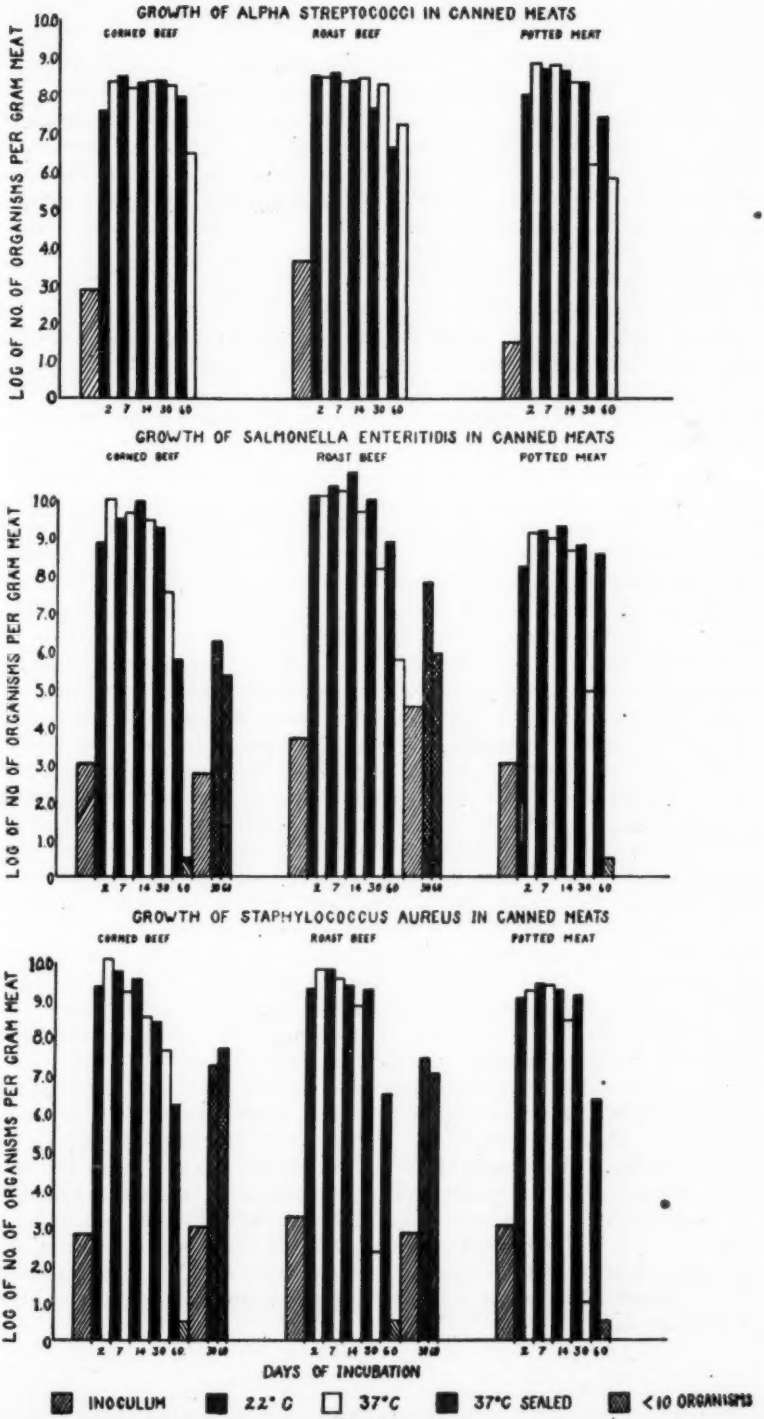
GROWTH OF ALPHA STREPTOCOCCI IN CANNED MEATS
CORNED BEEF
ROAST BEEF
POTTED MEAT
LOG OF NO. OF ORGANISMS PER GRAM MEAT
GROWTH OF SALMONELLA ENTERITIDIS IN CANNED MEATS
GROWTH OF STAPHYLOCOCCUS AUREUS IN CANNED MEATS
DAYS OF INCUBATION
INOCULUM
22° C
37°C
37°C SEALED
<10 ORGANISMS

FIG. 1.

and alemite gun were used through the courtesy of Dr. John Yesair of the National Canners Association). The tubes were weighed before and after filling and the weight of meat in each was determined by subtraction. Fifty-five tubes were filled in each experiment. Five tubes were opened immediately and the average inoculum per gram of meat was determined by bacterial plate counts. One-half of the remaining tubes were incubated at 22°C.(71.6°F.) and the others at 37°C.(98.6°F.). Bacterial counts of the contents of five tubes in each series were made at 2-, 7-, 14-, 30-, and 60-day intervals.

In making the bacterial counts, the contents of the tube were emulsified in sufficient sterile saline to give a 1:10 dilution. After thorough mixing decimal dilutions were made with saline, and veal infusion agar pour plates were made with one-ml. portions of appropriate dilutions. Five per cent sheep's blood was added to the media in the case of the alpha-type streptococci.

Since drying seemed to be a major factor in survival over the long time intervals, tubes inoculated with alpha-type streptococci were incubated in desiccator jars saturated with water vapor, and the 30- and 60-day intervals were repeated for *Staph. aureus* and *S. enteritidis* in corned beef and roast beef, using tubes sealed with paraffin to prevent evaporation.

Spread of *Staph. aureus* through contents of the can from a definite point of inoculation was studied by inoculating cans of potted meat, roast beef and corned beef at a definite point and determining the presence of staphylococci in different locations in the product. Cans of specially ground and untreated roast beef and corned beef were inoculated in the center of the top surface by depositing one drop of a 24-hour broth culture through a hole punched in the top of the can. After inoculation the can was resealed. Potted meat and specially ground corned beef and roast beef were inoculated at the center by removing a plug of meat with a cheese trier and introducing one drop of 24-hour broth culture at the base of the opening. The plug of meat was then replaced and the can resealed with a new sterilized lid, which was soldered over the opening. Incubation was carried out at 22 and 37°C. Cultures on veal infusion agar pour plates were made at intervals of 2, 7, 14, 30, and 60 days from the following areas of can contents: (1) center of the bottom surface, (2) center of the side surface, (3) at the junction of the top and side surfaces, (4) center of the top surface, (5) inside the plug of meat one-half inch from the bottom, (6) inside the plug of meat one-half inch from the side, (7) center of the plug of meat. Sampling was done by removing the entire block of meat through the end of the can opposite to that opened at the time of inoculation, after which samples were removed from the block by means of a flamed spatula. The sample of approximately 0.5 gram of meat was mixed with nine ml. of sterile saline and pour plates were made with one ml. of the suspension. Plates were examined for growth after 48 hours' incubation at 37°C. In the case of cans of untreated roast beef the liquid in the can was cultured, and the meat in direct contact with the liquid was sometimes omitted. One can of each product was sampled at each time interval at both 22 and 37°C.

TABLE 1

Spread of Staphylococcus Aureus to Areas of Can Contents From a Given Point of Inoculation

Type of meat product	Point of inoculation	Temperature of incubation (°C.)	Days[1] after inoculation cultures were positive—area cultured						
			Bottom surface	Bottom inside meat[2]	Side surface	Side inside meat[2]	Top surface (border)	Top surface (center)	Center
Ground corned beef	Center	22	7	<2	14	7	14	<2	<2
		37	7	<2	7	<2	<2	<2	<2
	Top surface	22	14	14	14	14	14	<2	14
		37	<2	<2	<2	<2	<2	<2	<2
Ground roast beef[3]	Center	22	<2	<2	7	<2	7	7	<2
		37	<2	<2	<2	<2	<2	<2	<2
	Top surface	22	<2	30	<2	7	<2	<2	<2
		37	<2	<2	<2	<2	<2	<2	<2
Potted meat	Center	22	30	30	30	<14	<14	<14	<14
		37	<7	14	<7	14	<7	<7	<7
Untreated corned beef	Top surface	22	30	30	30	7	7	<2	7
		37	14	14	14	14	7	<2	14
Untreated roast beef[3]	Top surface	22	<2	....	....	60	....	<2	60
		37	<2	....	....	60	....	<2	<30

[1]Cultures taken after 2, 7, 14, 30, and 60 days of incubation. [2]Cultures made one-half inch from surface. [3]Contents very moist with frank liquid in some cans.

RESULTS AND DISCUSSION

The organisms studied grew well in all three meat products (Fig. 1). The size of the inoculum varied from 30 to 5,000 organisms per gram of meat and the counts obtained after two days' incubation varied from 10×10^6 to 30×10^6. In general, growth was more rapid at 37°C. than at 22°C. Organisms survived for at least 60 days at 22°C. in all cases. At 37°C. drying of the contents of the tubes seemed to be a major factor determining survival, for when tubes were sealed with paraffin or incubated in an atmosphere saturated with water vapor, survival was good through the time of observation, a fact which is not surprising since Francis (1943) found survival of *Past. pestis* for 10 and 20 years in sealed tubes.

Within the limits of our methods of determination, spread of *Staph. aureus* throughout the contents of the can from a definite point of inoculation occurred in all instances. The spread was more rapid in roast beef than in the other meat products tested, a finding undoubtedly due to the greater amount of moisture in the can. Growth into the solid slabs of meat was not as rapid as that through the ground products. The time required for *Staph. aureus* to spread from a given point of inoculation to sample areas is shown (Table 1).

No abnormal odors were associated with the meats in any of the experiments. When extensive growth of *Staph. aureus* occurred, the yellow growth was often observed on the surface throughout the meat, and sometimes gave a "ropey" consistency to the meat products.

These results indicate that, in the event of contamination of the types of canned meat products studied, organisms rapidly become distributed throughout the entire contents of the can. Therefore, discarding only portions of the contents of a contaminated can would afford no protection against the bacteria. Likewise, survival of organisms for at least 60 days at both room and incubator temperatures indicates the possible danger of storage of contaminated products at these temperatures. That they probably would be viable for much longer periods is indicated by the work on plague bacilli referred to above.

SUMMARY

Staphylococcus aureus, *Salmonella enteritidis*, and alpha-type streptococci experimentally inoculated into test-tube preparations of canned roast beef, corned beef, and potted meat grew luxuriantly and survived for at least 60 days at 22 and 37°C. when loss of moisture was prevented.

Staph. aureus inoculated into cans of specially ground and untreated roast beef and corned beef at a definite point, either on the top surface or in the center of the can, spread rapidly throughout the contents of the can.

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COMPOSITION OF NORTHERN CALIFORNIA TOMATOES

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The work described in this paper was undertaken for the purpose of determining the variability of the composition of tomatoes from one season to another, from one locality to another in the same season, and from one grower to another in the same locality. It deals with the composition of tomatoes received at this plant from 1937 to 1943.

The Riverbank Canning Company is a large manufacturer of tomato paste, and since 1937, has received not less than 40,000 tons of tomatoes annually. Tomatoes are received from a radius of 200 miles north-south and 75 miles east-west.

METHODS OF ANALYSIS

The pH determinations were made with a Beckman glass electrode. Total acidity was determined in the clear juice using phenol-phthalein as indicator, according to A.O.A.C. methods (1940), XXVI, 24, page 341.

Total solids were determined by the refractometer method in the 1937-1940 period and by drying the samples at 70°C.(158°F.) and a pressure of 50 mm. for five hours in the 1941-1943 period. The five-gram samples were dried in a flat-bottom aluminum dish, 1½ inches deep and 2¾ inches in diameter.

Reducing sugars were determined by the tentative Lane-Eynon volumetric method, A.O.A.C. (1940), XXXLV, 32, page 498.

Ascorbic acid determinations were made by taking 100 grams of tomatoes, macerating them in a Waring blendor in the presence of 40 ml. of five per cent solution of metaphosphoric acid, adding another 40 ml. of metaphosphoric acid solution of the same strength, adding 10 ml. of five per cent bentonite and sufficient water to bring to 200 ml., and centrifuging. Ten ml. of the clear juice was titrated with a solution of sodium 2,6-dichlorobenzenoneindophenol (containing 50 mg. of dye per 100 ml. of solution) to a pink end point that lasts about 10 seconds.

The dye solution was prepared by treating 250 mg. with ether until no further color was extracted, then dissolving it in hot distilled water and filtering into a 500-ml. volumetric flask. The filter paper was then washed free of blue color with hot distilled water. The standardization, according to Menaker and Guerrant (1938), was made by taking 10 ml. of the dye solution, adding one gram of potassium iodide, 0.50 to one ml. of 1:4 sulfuric acid (one part of concentrated sulfuric acid to four parts of distilled water), and shaking. The liberated iodine, to which 0.50 ml. of starch solution had been added, was titrated with 0.01 normal solution of sodium thiosulfate. One milliliter of the 0.01 normal sodium thiosulfate is equivalent to 0.88 mg. of ascorbic acid.

RESULTS AND DISCUSSION

In the 1937-1940 period, total solids determinations were made by taking an average of four pounds of tomatoes for each lot of about 10 to 20 tons of tomatoes received at this plant. During this period the tomatoes received were of the San Marzano variety (pear-shaped) except the ones grown in the Merced area which were of the small, round varieties commonly used for paste. In the 1941-1943 period, the tomatoes received were of the San Marzano and the round varieties: improved Pearson, Santa Clara, Pritchard, and others. All analyses were made by using the official methods of the A.O.A.C. on composite samples of the San Marzano and round tomatoes.

The maximum, minimum, and average percentages of total solids from various localities determined by the refractometer method are shown (Table 1). The maximum and minimum represent individual grower averages, and the average is the total average for that locality. From 1937 to 1940 more than 8,000 samples were analyzed by this method. The weekly average percentages of total solids for the period 1937-1940 are shown (Fig. 1). The weekly average pH and the per cent of total solids and total acids for 1941, also the pH and the per cent of total solids, reducing sugars, and total acids for 1942 are shown (Fig. 2). The pH and the per cent of total solids, reducing sugars, and total acids and also the milligrams of ascorbic acid per 100 grams of tomatoes for 1943 are shown (Fig. 3). Determinations of tomatoes of the improved Pearson variety picked at different stages of maturity during September 1943 (Table 2) are from the same vine grown in the Modesto area.

Total Solids: The total solids contents of tomatoes from 26 different localities (Table 1) show averages for 1937, 1938, 1939, and 1940 of 6.2, 6.8, 6.5, and 6.4, respectively. Most of the tomato samples were taken from the localities named, but during these four years tomatoes from 52 different localities were analyzed for total solids.

The year 1938 has the highest percentage of solids, and this is probably due to very suitable climatic conditions that existed that year for the growth of good-quality tomatoes. The maximum and minimum percentages of solids found during these four years is 7.8 and 5.1, respectively. In some localities, such as Stockton and Modesto, a variation of about one and one-half per cent between the maximum and minimum was found; while in others, such as Tracy and Walnut Grove, the variation between the maximum and minimum was less than one-half of one per cent. The reason for the wide variation between the maximum and minimum may be due to soil conditions or to methods of cultivation and irrigation used by different growers.

The total solids contents were low in July and August, started to increase in the latter part of August, reached a maximum during September 20 to October 10, and after October 22, definitely began to decrease and, in some cases, to reach a lower point than in July or August (Figs. 1, 2, and 3). On September 24, 1937, the average of total solids was found to be less than on August 21, 1937, while on July 23, 1938, it was higher than on August 13, 1938.

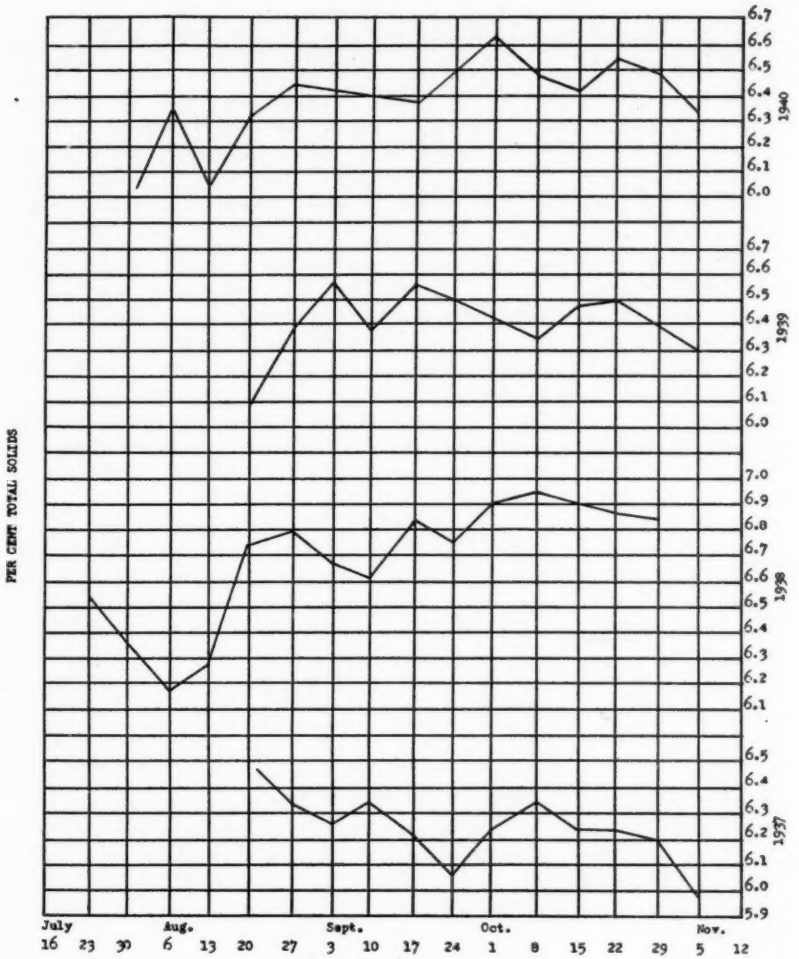

FIG. 1. Weekly average.

The average total solids for 1941, 1942, and 1943 were 6.32, 5.68, and 5.60 for the San Marzano and 6.20, 5.47, and 5.51 for the round varieties. The maximum and minimum total solids for the 1941-1943 period were 6.82 and 4.44 for the pear-shaped tomatoes and 7.04 and 4.40 for the round tomatoes. The season variation of the total solids is comparable to the previous four years. The reason for the low average of total solids found in the 1942 and 1943 seasons may be attributed to the fact that the average temperature for these two years was below normal.

In our study we found that the total solids of the tomatoes was higher after prolonged warm, dry weather during harvesting time (September 10 to October 10) and was lower after a rainy period. These findings are in

TABLE 1

Total Solids of Tomatoes

Locality	1937				1938				1939				1940				Four-year average
	Number of samples	Maximum	Minimum	Average	Number of samples	Maximum	Minimum	Average	Number of samples	Maximum	Minimum	Average	Number of samples	Maximum	Minimum	Average	
Suisun[1]	9	6.7	6.0	6.2	38	7.0	6.2	6.9	32	6.9	6.8	6.8	....	....	....	....	6.8
Empire[1]	5	6.6	6.5	6.6	2	7.1	6.8	6.9	....	....	....	....	3	6.9	6.4	6.7	6.7
Tracy	50	6.8	6.4	6.6	145	72.	7.0	7.1	84	6.7	6.0	6.4	250	6.7	6.1	6.6	6.7
Ripon	16	7.1	5.8	6.4	65	7.1	6.6	6.8	105	6.9	6.4	6.7	80	6.9	6.4	6.6	6.7
Modesto	120	7.3	5.9	6.2	125	7.3	5.9	6.9	270	7.1	5.6	6.8	101	6.9	5.9	6.5	6.7
Ceres[1]	4	6.5	5.8	6.3	2	8.0	7.8	7.9	6	6.9	6.5	6.6	....	....	....	....	6.7
Sims Station	65	6.5	6.1	6.3	90	7.0	6.9	6.9	102	6.8	6.4	6.7	85	6.6	6.5	6.5	6.6
Lathrop[1]	....	....	....	....	7	7.2	6.8	7.0	28	6.9	6.4	6.6	35	6.7	6.3	6.4	6.6
Walnut Grove[1]	....	....	....	....	105	7.0	6.8	6.9	180	6.6	6.4	6.6	200	6.8	6.1	6.5	6.6
Escalon	50	6.7	5.6	6.1	65	7.0	6.7	6.8	84	7.0	5.7	6.6	50	6.9	6.3	6.7	6.6
Lodi	10	6.9	6.0	6.4	60	7.4	6.4	6.9	220	6.7	6.4	6.6	200	6.7	5.8	6.5	6.6
Sacramento	210	6.2	5.8	6.3	200	7.4	6.3	6.9	220	6.7	6.1	6.5	44	6.8	6.5	6.6	6.6
Stockton	240	7.0	5.1	6.3	450	7.4	6.3	6.8	540	7.3	6.0	6.5	360	7.8	5.6	6.4	6.5
Courtland[1]	....	....	....	....	81	6.7	6.1	6.6	35	6.7	6.1	6.2	200	7.1	5.9	6.5	6.5
Manteca	80	6.3	6.0	6.1	75	7.5	6.6	7.0	30	6.9	6.5	6.8	150	6.8	5.4	6.4	6.5
Salida	35	6.8	5.9	6.2	20	7.2	6.8	7.1	25	6.7	6.1	6.5	21	6.8	6.3	6.4	6.5
Oakdale	40	6.8	5.9	6.2	75	7.2	6.5	6.7	120	7.5	6.2	6.4	110	6.9	6.0	6.4	6.5
Clements[1]	42	6.4	6.1	6.2	35	6.7	6.2	6.4	50	6.9	6.4	6.6	....	....	....	....	6.4
Thornton	8	6.2	5.7	5.8	18	6.9	6.1	6.5	25	6.6	6.5	6.5	75	6.5	6.2	6.4	6.4
Acampo	35	6.3	5.7	6.2	25	7.0	6.6	6.9	125	6.6	6.1	6.4	180	6.7	6.4	6.4	6.4
Isleton[1]	....	....	....	....	20	6.8	6.6	6.7	15	6.5	6.0	6.3	60	6.4	6.2	6.3	6.4
Merced	50	7.0	6.0	6.2	230	7.3	6.5	6.5	45	6.7	6.6	6.6	180	6.6	6.2	6.2	6.4
Linden	20	6.2	6.1	6.1	40	6.8	6.3	6.7	65	6.6	6.2	6.4	35	6.6	6.0	6.1	6.4
Turlock[1]	....	....	....	....	9	6.7	6.4	6.5	15	6.5	6.2	6.4	5	6.3	6.0	6.2	6.3
Yuba City	35	6.3	5.9	6.1	8	6.9	6.5	6.7	40	6.6	6.0	6.1	10	6.7	6.5	6.6	6.2
Verona	100	6.4	5.8	6.1	40	6.6	6.3	6.6	30	5.6	5.1	5.3	75	6.7	6.3	6.5	6.2
Average of all localities	1224	....	....	6.2	1967	....	....	6.8	2491	....	....	6.5	2509	....	....	6.4	6.5

[1]Represents three years' average.

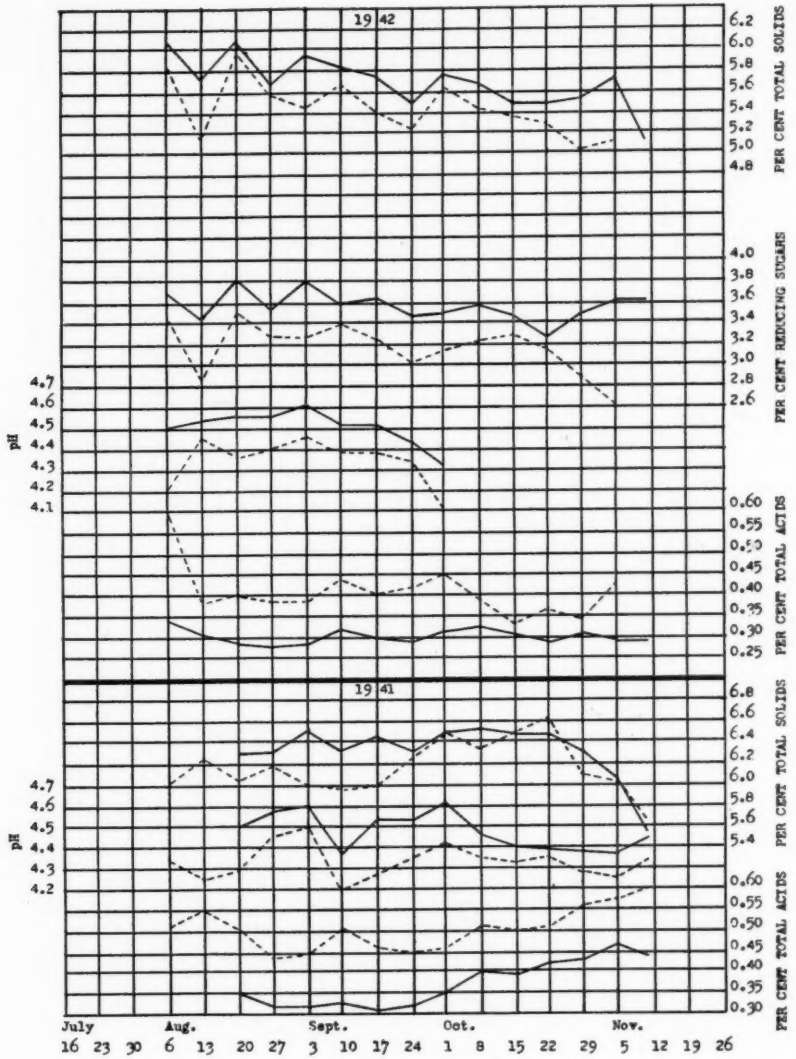

FIG. 2. Weekly average.

———— San Marzano tomatoes. — — — — Round tomatoes

agreement with those reported by Brooks and MacGillivray (1928). Saywell and Cruess (1933) found that the total solids of the tomatoes picked early or late in the season is lower than in the middle, but according to them, these changes are not sharply defined. Their findings are in agreement with those reported in this paper.

Effect of Locality: The average total solids of tomatoes found in the localities examined shows no marked variation, except those (Verona, Yuba

City) situated north of Sacramento, which are subjected to a much hotter, dry summer climate than the others. Saywell and Cruess found that the total solids of tomatoes grown in the cool northern California climate are much higher than in the hot, dry southern California climate.

pH: The average pH of the San Marzano tomatoes for the 1941-1943 period was 4.47, the maximum 4.75, and the minimum 4.01; while for the round tomatoes the average was 4.32 with a maximum of 4.69 and a minimum of 3.98. Generally, higher pH was accompanied by low acidity and lower pH by a high acidity.

Total Acidity:[1] The average acidity of the San Marzano was found to be 0.30 with a maximum of 0.49 and a minimum of 0.23, against an average of 0.43 with a maximum of 0.70 and a minimum of 0.25 for the round tomatoes.

Ratio Total Solids to Acid: The ratio of total solids to acid is higher in the San Marzano than in the round tomatoes (Figs. 2 and 3).

Reducing Sugars: We find that the reducing sugars are higher in the pear-shaped than in the round tomatoes. The 1942 and 1943 averages for the pear-shaped are 3.57 and 3.45 and for the round tomatoes, 3.34 and 3.19, respectively. During these two years the maximum and minimum are 4.20 and 2.52 for the former and 3.96 and 2.43 for the latter. Saywell and Cruess (1933) found, by analyzing over 240 samples of tomatoes, a minimum of 2.70 and a maximum of 4.30 with an average range of 3.30 to 3.50 of total reducing sugars for the 1926, 1928, 1929, and 1931 crops. Bohart (1940) reports 3.76 as the average total reducing sugars of 48 samples of western tomatoes, with a minimum of 2.57 and a maximum of 5.36.

Ratio of Sugar to Acid: The ratio of sugar to acid is higher in the pear-shaped than in the round tomatoes (Figs. 2 and 3).

Ascorbic Acid: The average amount of ascorbic acid found in the pear-shaped and round tomatoes is 31.1 and 30.3 mg. per 100 grams of tomatoes, respectively. The maximum and minimum amounts of ascorbic acid found are 45.6 and 19.2 mg., 41.3 and 19.8 mg. per 100 grams of pear-shaped and round tomatoes, respectively.

SUMMARY AND CONCLUSION

Generally, it was found that the higher the total solids of tomatoes the higher were also the reducing sugars, the ascorbic acid content, and the pH, and the lower the acidity. The highest total solids was found about the middle of the season or during the period of September 20 to October 10; while the lowest was found at the beginning and end of the season and definitely started to decrease after October 22 (Figs. 1, 2, and 3). The highest ascorbic acid content was found to be near and before the ripening state and lowest in green or overripe tomatoes (Table 2).

Pear-Shaped Tomatoes: The average total solids ranged from 5.60 to 6.83 per cent, the pH from 4.43 to 4.52, the acidity from 0.33 to 0.37 per cent, the reducing sugars from 3.45 to 3.57 per cent, and the ascorbic acid content averaged 31.1 mg. per 100 grams of tomatoes.

[1]Total acidity as anhydrous citric acid.

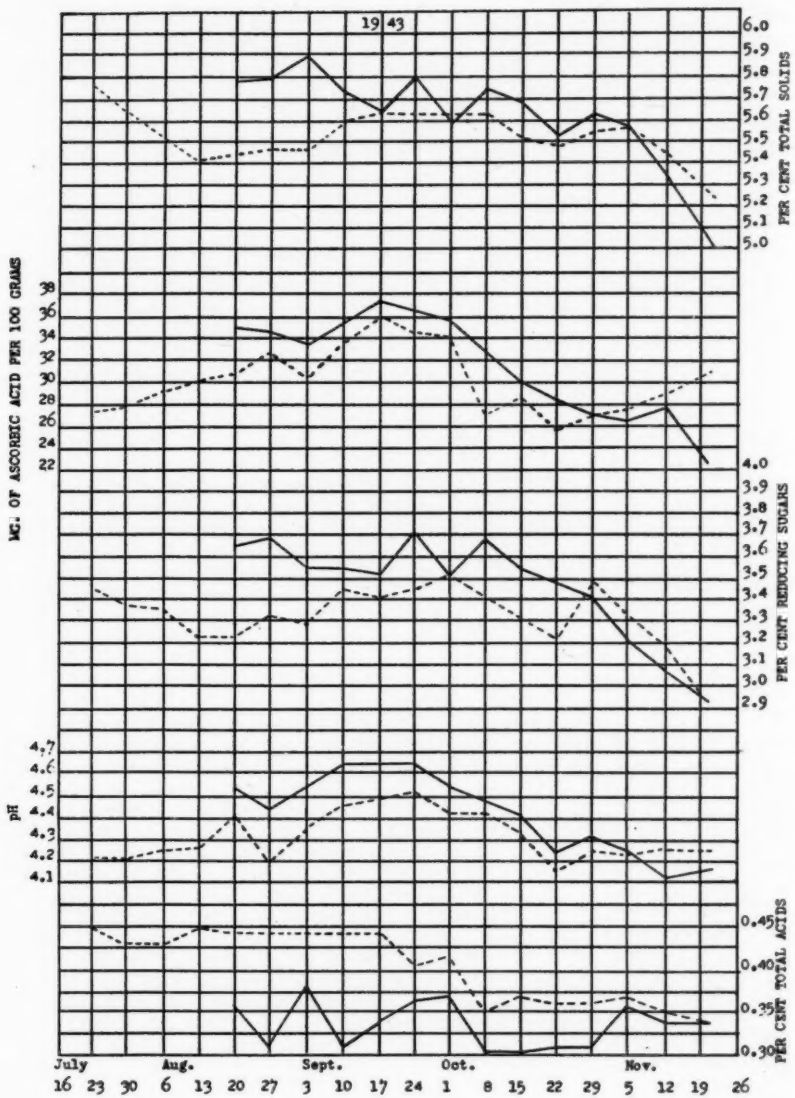

FIG. 3. Weekly average.

———— San Marzano tomatoes. — — — — Round tomatoes

Round Tomatoes: The average total solids ranged from 5.47 to 6.20 per cent, the pH from 4.31 to 4.32, the acidity from 0.40 to 0.50, the reducing sugars from 3.19 to 3.34 per cent, and the ascorbic acid content averaged 30.3 mg. per 100 grams of tomatoes.

The average total solids, reducing sugars, and the pH were found to be higher in the San Marzano than in the round tomatoes; while the total acidity is lower in the former than in the latter variety. No appreciable difference was found in the average ascorbic acid content between the pear-shaped and the round tomatoes (Fig. 3).

TABLE 2

Analysis of Improved Pearson, Picked From Same Vine at Different Stages of Maturity

Constituents	Very green	Green	Pink	Pinkish-red	Red	Over-ripe
pH	3.90	3.90	3.92	4.40	4.03	4.58
Total acidity as anhydrous citric acid	0.61	0.50	0.41	0.38	0.33	0.37
Total solids	5.44	5.50	4.86	5.76	4.61	4.46
Soluble solids	4.06	3.86	3.81	4.63	3.64	3.68
Insoluble solids[1]	1.43	1.70	1.10	1.19	1.01	0.81
Reducing sugars	2.10	2.38	2.37	2.70	2.44	2.03
Ratio sugars to acids	3.40	4.70	4.80	7.10	7.40	5.50
Sugar coefficient	38.73	43.27	48.78	46.70	52.90	45.50
Ascorbic acid per 100 grams (mg.)	17.70	17.20	30.60	26.40	33.20	21.60

[1]Insoluble solids were determined according to the formula of W. F. Ebers of the National Canners Association Laboratories, Bohart (1940).

The composition of tomatoes varies from season to season and from one locality to another in the same season owing to climatic conditions. Localities with a hot, dry climate have a lower average of total solids than those with a milder one. The variation from one grower to another in the same locality may be due to soil conditions and to different methods of cultivation and irrigation used by different growers.

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BEHAVIOR OF PATHOGENIC BACTERIA IN FERMENTED MILKS

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Fermented milk as a carrier of pathogenic organisms has been of considerable concern to milk sanitarians. Until recently little evidence was available to indicate that acid milk products were media for, or carriers of, pathogenic organisms. However, in summaries of milk-borne epidemics issued by the Public Health Service, Frank (1940) and Fuchs (1941) noted three outbreaks involving 106 cases attributed to buttermilk. It seems wise, therefore, to obtain as complete information as possible on the behavior of pathogens in fermented milks. Possibilities of infection through fermented milks are discussed in this report, and suggestions are made for safeguarding the health of consumers of these products.

REVIEW OF LITERATURE

Several investigators reported rapid destruction of pathogenic microorganisms in acidified milk preparations. Seitz (1886) was one of the first to study development of *Eberthella typhosa* in nonsterile milk. He could not detect viable organisms two days after adding large numbers of these organisms to milk. According to Lazarus (1890) neither *Eberthella typhosa* nor *Vibrio comma* grew after 24 to 36 hours in strongly acid or already curdled milk. High acidity played a prominent role in rapid disappearance of *Vibrio comma*. That *Vibrio comma* died when milk became sour was also reported by Schrank (1895); contrary to observations of Weigmann and Zirn, he concluded that progressive acidity of the milk rather than competition with milk bacteria caused death of *Vibrio comma* in sour milk. At room temperature, according to Fraenkel and Kister (1898), *Eberthella typhosa* remained viable for three days in buttermilk, while at incubator temperatures they died after 24 hours. This was the observation of Rubinstein (1902) also. As a result of experiments by Bassenge (1903) *Eberthella typhosa* remained viable for five days in raw milk with 0.27 per cent acidity and after six days with 0.36 per cent acidity, but within 24 hours with 0.63 per cent acidity no viable cells could be demonstrated. *Salmonella schottmülleri* was found by Broers (1904) to survive longer in buttermilk than *Eberthella typhosa*. Broers (1905) could not demonstrate viable *Eberthella typhosa* in buttermilk resulting from manufacture of butter from sour milk infected with this organism. The following year Broers and Ten Sande (1906) compared durability of *Mycobacterium tuberculosis* and *Eberthella typhosa* in kefir, and found the latter unable to survive the process of kefir preparation. On the other hand, *Mycobacterium tuberculosis* flourished with undiminished virulence. *Eberthella typhosa* would not develop in a medium with milk bacilli, according to results of Rosenthal (1906). *Lactobacillus bulgaricus* inoculated with *Corynebacterium diphtheriae* would get the upper hand and halt its devel-

opment within two days. Behla (1905-8) noted that when milk, buttermilk, etc. reached 0.4 per cent lactic acid, *Eberthella typhosa* was killed within 24 hours. Cannata and Mitra (1911) observed that *Lactobacillus bulgaricus* and other lactobacilli had a remarkable antibacterial effect upon *Eberthella typhosa, Salmonella paratyphi, Salmonella schottmülleri, Shigella dysenteriae,* and *Staphylococcus aureus.* They believed that the antibacterial properties of the organism did not depend upon production of lactic, formic, or acetic acid since these were present in such slight amounts that they could not restrict development of pathogenic organisms. Northrup (1911) tested the action of *Eberthella typhosa* when in contact with milk acidified by cultivation of lactic acid bacteria and filtered through a Chamberland filter. Acids produced by *Streptococcus lactis* and *Lactobacillus bulgaricus* varied in degree of germicidal action. Northrup considered possible factors in uncontrolled natural conditions which would alter results. If certain species of microorganisms were present in milk, they might, either by growing in association with lactic acid bacteria or by producing metabolic products of their own, cause more rapid destruction of *Eberthella typhosa* than the lactic acid-producing bacteria were capable of causing alone. On the contrary certain species of bacteria might predominate which would check production of acid by the lactic bacteria or use it themselves or perhaps even live in symbiotic relationship with *Eberthella typhosa.* It is probable that some of these conditions occur, since milk infected with *Eberthella typhosa* must have been subjected to sources of contamination from which a varied flora would be acquired.

An outstanding contribution was made by Heinemann (1915) who studied the germicidal effect of lactic acid in milk. Using progressive amounts of lactic acid in sterile milk, he determined the acid tolerance of six pathogenic microorganisms. The following limits of lactic acid tolerance were noted:

Escherichia coli 0.58-0.60 per cent
Shigella paradysenteriae Flexner 0.3-0.4 per cent
Eberthella typhosa 0.3-0.4 per cent
Salmonella schottmülleri 0.28-0.37 per cent
Corynebacterium diphtheriae Park 8 0.27-0.32 per cent
Vibrio comma 0.0 per cent.

Vibrio comma would not grow in acid media. Buttermilk usually contains 0.6-0.9 per cent acid, so it would seem the product could be considered safe even though the original milk had been contaminated with pathogenic bacteria. Heinemann found, however, that the slower milk soured, the greater the danger of survival of pathogenic microorganisms since acid-tolerant strains would have a better chance to grow. He concluded that buttermilk should be looked upon with suspicion, especially if heavily contaminated, unless prepared from pasteurized milk. Trillat and Fouassier (1916) obtained similar results with lactic acid added to milk containing *Eberthella typhosa*; lactic coagulation was believed to be unfavorable for reproduction of these organisms.

Bindseil (1917) found *Eberthella typhosa* short lived in buttermilk. In yoghurt made from infected raw milk *Eberthella typhosa* perished

within 24 hours and could not be demonstrated after 48 hours in kefir.

Ohta (1917) and Field (1924) added portions of mixed stool bacteria of infants to samples of milk acidified to pH 4.0 and obtained sterile cultures from them after incubation at room temperature over night. Similar results were obtained by Marriott and Davidson (1923) who proposed acidified whole milk as a routine infant food.

That *Eberthella typhosa* was sensitive to acidity and was usually destroyed in milk acidified to the same degree as fresh buttermilk in the New York market was indicated by experiments performed by Marsh (1918). Buttermilk would seem to be free of danger as a means of distributing *Eberthella typhosa, Salmonella paratyphi,* or *Shigella dysenteriae* in the ordinary course of events. Mosse and Happe (1927) observed comparable results while testing hemolytic streptococci, enterococci, and *Eberthella typhosa* in a lactic acid-milk mixture.

Carpenter and Boak (1928) inoculated cream with *Brucella abortus*; it failed to infect guinea pigs when the pH reached 5.0. Undoubtedly death of *Brucella abortus* was hastened by lactic acid production. *Brucella abortus* cultivated in milk at the same time as a lactic ferment was killed by the acidity of the medium in less than two days, according to results of research by Mazé and Césari (1931). They believed that lactic fermentation suppressed all danger of contamination for the fresh fabricated cheese industry. Doldi (1935) reported that milk serum following acid fermentation to a concentration of one per cent lactic acid stopped development of *Eberthella typhosa, Salmonella paratyphi,* and *Shigella dysenteriae.* In the neutralized milk serum *Salmonella paratyphi* and *Escherichia coli* developed only slightly; inhibition appeared not to depend entirely on lactic acid content. The experiments of Heinemann were for the most part verified by Fügner (1935). A striking departure from the general results occurred with concentration of two to three per cent lactic acid in which the intestinal pathogens with the exception of *Vibrio comma* were able to grow. In raw milk Kredba (1935) observed *Eberthella typhosa* dying in two or three days at 37°C.(98.6°F.), while in the presence of *Lactobacillus bulgaricus* this phenomenon occurred in 24 hours. Kliewe and Hsü (1935) also studied the action of *Eberthella typhosa* in sour milk. Raw milk samples with pH 5.02 were infected with *Eberthella typhosa* and incubated at 37°C. After 24 hours the pH value had changed to 3.56 and *Eberthella typhosa* was dead. *Salmonella paratyphi* and *Salmonella schottmülleri* were not killed in 11 days at pH 5.04-4.84. Kliewe and Hsü believed that acidity of sour milk played no decisive role as cause of death, for the germs were not killed when a high degree of acidity was obtained. The effect of lactic acid added to broth cultures of pathogenic organisms was noted by Voille (1936). Although the experimental conditions were not practical, it is worth while to consider the results. *Brucella abortus, Eberthella typhosa,* and *Mycobacterium tuberculosis* were killed in less than 24 hours by 0.5 per cent lactic acid in broth, and in less than 24 hours by 0.1 per cent.

Sircana's (1937) work with *Eberthella typhosa* in yoghurt was verified by reports of Roman (1939) who, from the viewpoint of epidemiology, found acid milks to be more satisfactory than fresh milk in preventing

development of *Eberthella typhosa, Salmonella schottmülleri,* and *Shigella paradysenteriae* Flexner. The acidity of the milk was considered more important in its inhibitive action than the antagonistic effect of the microorganisms producing acid. Similar conclusions were also reached by Kazberyuk (1940), who observed the influence of kumiss, mare's milk inoculated with *Lactobacillus bulgaricus,* on an organism called *B. paratyphi abortus equi.*

That intestinal pathogens were able to last from one to three days in fermented milks was reported by Lemmin (1906), Chatterjee (1910), Kaiser (1921), and Honda (1938). Lemmin churned milk two or three days after infecting it with *Eberthella typhosa,* and could not isolate living cells from buttermilk. A lactic acid-producing *Streptothrix* was isolated by Chatterjee from dadhi, a fermented milk product in India. One-day-old cultures of pathogens were introduced into pure 24-hour cultures of the *Streptothrix.* After 24 hours at 37°C. *Vibrio comma* and *Corynebacterium diphtheriae* had completely disappeared. *Escherichia coli, Shigella dysenteriae, Eberthella typhosa,* and *Salmonella schottmülleri* could be found up to, but not beyond, 48 hours. Kaiser observed that different strains of *Salmonella* organisms reacted differently to lactic acid in milk. Time varied from 12 to 72 hours before these organisms died in a lactic acid concentration of "4 Prom." The death of *Salmonella* organisms in yoghurt occurred with a lactic acid content of 1.5-2.0 per cent between 48 and 72 hours. There were strains that could tolerate a higher acid content for 84 to 120 hours. Honda concluded that *Eberthella typhosa* and *Salmonella paratyphi* died two or three days after mixing them with sour milk. Krumwiede and Noble (1914) observed *Eberthella typhosa* to be gradually killed in sour cream by the acids produced, the rate of destruction being proportional to degree of acidity and number of *Eberthella typhosa* present. The apparent disappearance of *Eberthella typhosa* in sour cream with a natural flora was due in part to overgrowth by these bacteria and in part to difficulty of isolation, by available methods, of the proportionately few *Eberthella typhosa* remaining. With moderate contamination *Eberthella typhosa* was killed in about four days. Since Fischer (1930) demonstrated, at 12 and 37°C.(53.6 and 98.6°F.), living *Salmonella schottmülleri* for three or four days in souring milk, he recommended that milk be pasteurized previous to addition of *Streptococcus lactis* cultures. At room temperature the organisms remained viable for one month. Fischer reviewed experiments by von Drigalski, who found no multiplication of *Eberthella typhosa* in buttermilk which was neutralized with three to six cubic centimeters of 0.1 normal sodium hydroxide per 100 cubic centimeters of milk. A significant contribution to our knowledge concerning the activity of *Eberthella typhosa* in sour milk was made by Bohdanowicz (1930). The role of coagulated milk in the epidemiology of typhoid fever was believed to be important, particularly in Poland where consumption of fermented milk was common to all classes of people. It was found that at pH 4.4 *Eberthella typhosa* could live in sterilized, curdled milk for five days. Acidity of market curdled milk in which *Eberthella typhosa* could not be shown to be viable after three days was at least pH 4.4 *Streptococcus lactis* exercised an antagonistic action upon *Eberthella typhosa*

depending uniquely upon the changes in acidity produced as a result of fermentative activity of the streptococci. Bohdanowicz concluded that coagulated milk might have been a source of typhoid fever infection and, as such, should be subjected to sanitary control measures. Rosenthal (1910) found the "bacille d'Achalme, variéte rhumatismale" to be destroyed within three to seven days when mixed with *Lactobacillus bulgaricus*.

Sterilized milk of weak acidity constituted an excellent culture medium, according to Kliewe and Eldracher (1935). When the acidity reached 17-20 degrees (using the method of Thörner and Pfeiffer) *Eberthella typhosa* died in 24 hours at 37°C., although *Salmonella schottmülleri* resisted for one to two weeks. At 20°C.(68°F.) *Eberthella typhosa* as well as *Salmonella schotmülleri* survived for many weeks, even when the acidity was raised to 33 degrees. In the destruction of pathogenic intestinal bacteria in raw sour milk the presence of bacteria of the coliform group were of decisive significance. When the bacteria of the coliform group were present, the pathogenic organisms, especially *Eberthella typhosa*, were destroyed within a few hours or days, although *Salmonella schottmülleri* was somewhat more resistant. If the coliform group organisms were absent, the pathogenic bacteria were killed only by a higher temperature (37°C.), for then just the acid already formed exercised a germicidal power. Kliewe and Eldracher concluded that consumption of sour milk, buttermilk, etc. should be done with caution. They also mentioned a previous report by Hansen in which *Eberthella typhosa* was said to have been demonstrated after four weeks in 1.85 per cent lactic acid. Several investigators, including Cautley (1896-7), Bruck (1903), Darbois (1911), Wedemann (1925), Drescher and Hopfengärtner (1930), Lerche (1930), Stockmayer (1935), Kliewe and Schuppener (1937), and Steuer (1941), have been able to demonstrate viable pathogenic bacteria for over seven days in fermented milks. Cautley observed living *Eberthella typhosa* for several days after addition in large amounts to milk, and believed viability of these organisms depended on number and character of contaminating bacteria. Bruck endeavored to approximate natural conditions and recovered *Eberthella typhosa* from sour cream for 10 days after its separation from artificially infected milk. He also isolated them from strongly acid buttermilk for the same period of time. *Brucella melitensis* resisted action of lactic acid in milk as well as and even better than *Mycobacterium tuberculosis*, according to results obtained by Darbois. Whey and coagulated milk made from contaminated milk might have contained *Brucella melitensis* in a living state during a three-week period and were suspected of having caused infections during that time. Experiments performed by Wedemann showed that *Eberthella typhosa* and *Salmonella sp.* resisted the action of sour milk for several days so as to make possible an infection following consumption of such a product. *Brucella abortus* artificially inoculated into fermented milks was found viable in sour milk and yoghurt for eight days, in kefir up to 18 days, and in sweet and sour cream buttermilk for 30- and 9-day periods, respectively, according to investigations by Drescher and Hopfengärtner. Lerche incriminated lactic acid formation rather than lactic acid or other saprophytic bacteria as cause of death of *Brucella abortus* in sour milk. The speed of lactic acid pro-

duction was comparable to rate of increase of these organisms. Under pH 3.7 *Brucella abortus* was killed in one day, while between pH 3.9 and 3.7 a few of the organisms survived one to two days. Acidity denoted by pH 4.0 to 3.9 was tolerated for several days, pH 5.4 to 4.2 for many weeks, and pH 6.7 to 6.1 as long as several months. In approximately seven per cent of experimental cases *Brucella abortus* was isolated from buttermilk seeded with these organisms, and in one instance *Brucella abortus* was demonstrated in a commercial sample. Interesting observations in a related field of study were made by Stockmayer (1935). Milk from cows eliminating *Brucella abortus* through the udders was allowed to stand, and gravity cream was skimmed and churned. Plate counts were made on gentian violet malachite green media to determine the number of *Brucella abortus* in the various stages. Gravity cream was much richer in organisms than whole milk, while skim milk contained very few of the organisms. After churning, buttermilk retained most bacteria and few remained in the butter. Length of life of *Mycobacterium tuberculosis* in sour milk was observed by Kliewe and Schuppener. In contrast to Voille's prediction, sour milk with pH value 4.0-5.0 contained viable human-type *Mycobacterium tuberculosis* at least seven days after artificial inoculation. Bovine *Mycobacterium tuberculosis* was demonstrated viable after 20 days by animal inoculation. Steuer (1941) noted survival of *Salmonella paratyphi, Salmonella schottmülleri, Shigella dysenteriae,* and *Shigella paradysenteriae* for one to 15 days in buttermilk. The factors involved were temperature, acidity, and bacterial flora of the milk.

The following literature reviews contain accounts of little or no inhibition of pathogenic microorganisms by acid milk products. After exceedingly heavy seeding of *Eberthella typhosa* cells into raw milk, Heim (1889) demonstrated viable organisms for 21 to 35 days. The milk became sour after two days and curdled after four days at 13 to 18°C. (55.4 to 64.4°F.). Longevity of *Vibrio comma* was related to acid content, destruction being greater in acid milks. They remained viable for one day at room temperature and four days at cool temperature. Bolley and Field (1898) obtained surviving *Eberthella typhosa* for five days to one month from sour milk, and for two days to three months from buttermilk. *Eberthella typhosa,* according to Barthel (1901), increased in milk and remained alive for 25 days without a decrease in number owing to the formation of acid in the milk and, further, they themselves contributed to this acid production. A contribution to our knowledge concerning the durability of *Salmonella schottmülleri* in milk was made by Kersten (1909). He isolated these organisms, using malachite green agar plates, for 61 days at cool temperature, 64 days at room temperature, and four and one-half months at 37°C. after inoculating them into raw trade milk. All of the milk samples were sour after three days. Potter (1909-10) noted the effect of *Lactobacillus bulgaricus* and of *Streptococcus lactis* upon *Eberthella typhosa* in milk. Using Endo's agar medium she was able to show an increase of *Eberthella typhosa* up to 12 days at 8 to 12°C.(46.4 to 53.6°F.) and up to three days at 20°C.(68°F.). After eight hours at 37°C. *Eberthella typhosa* could be found in two out of seven cases. Potter concluded that repressing influence of *Lactobacillus bulgaricus* and *Streptococcus*

lactis upon growth of *Eberthella typhosa* was so slight as to be of no considerable significance. Along a different line of investigation Smith (1924) gave *Lactobacillus acidophilus* to typhoid and paratyphoid carriers, and observed that excretion of *Eberthella typhosa* and the paratyphoids was not influenced by acidophilus milk administration, although *Escherichia coli* was suppressed to a great extent.

Rimpau (1935) reported sour milk products, such as yoghurt and kefir, harmless when prepared from pasteurized milk. The importance of milk soured by lactic acid bacilli was judged differently, however. Certainly the acid-producing microbes would damage the pathogens to some extent, but with slow souring of milk many pathogens, such as *Eberthella typhosa,* would have time to multiply. The lactic acid may have been partially bound or decomposed so that actual acidity would not be enough to kill acid-susceptible organisms.

A notable contribution to the practical problems involved in the fermented milk industry was made by Kazaryan (1936). Lactic acid 0.1 per cent exerted a bacteriostatic effect on growth of pathogenic microbes of *Eberthella, Salmonella,* and *Shigella* groups, while greatest resistance was shown by *Salmonella schottmülleri.* Simultaneous cultivation of these organisms and *Lactobacillus caucasicus* did not hinder the former. Life span of microbes in mazun, a sour-milk preparation resembling acidophilus milk, was dependent upon accumulation of lactic acid and microbe concentration. Studies on white mice showed virulence of *Salmonella schottmülleri* and *Eberthella typhosa* unchanged after 18 to 24 hours, indicating that pathogenic bacteria were capable of multiplying in acidic milk products. Owing to wide use of these products, the possibility that it might have acted in spreading disease was of epidemiological significance.

Recently investigators have become especially conscious of the role of Staphylococci as causes of food poisoning. Ghosh, Bose, and Mukherjee (1938) found Streptococci and Staphylococci in most samples of dahi collected from vendors in Calcutta. It was difficult to decide whether these contaminations arose from street dust or were in the milk from which dahi was prepared. This point was of great importance from the public-health point of view. They introduced cultures of Streptococci, Staphylococci, and *Eberthella typhosa* into dahi preparations, and found only a few samples of dahi which could inhibit the pathogens. The following account by Chason and Waite (1942) of an outbreak of gastroenteritis was of interest because buttermilk was the vehicle for transmission of the toxin causing the illness. From homemade buttermilk a pure culture of *Staphylococcus aureus* was isolated which, grown under proper conditions, produced an enterotoxin demonstrable by the Dolman kitten test.

Bacterial cultures used in this work were obtained from culture collections of the Departments of Bacteriology and Dairy Bacteriology, University of Illinois. *Eberthella typhosa, Salmonella paratyphi, Shigella paradysenteriae* Flexner, *Salmonella schottmülleri,* and *Shigella dysenteriae* Shiga were previously sent from the University of Illinois College of Medicine; the first three had been obtained from the American Type Culture Collection. The lactic acid-producing bacteria obtained from Dr. M. J. Prucha, formerly of the Department of Dairy Husbandry, were

Lactobacillus acidophilus X, 4, and 12. Three cultures from a commercial product, Flav-o-lac Flakes, Dairy Laboratories, Philadelphia, Pennsylvania, were designated as "Acidophilus A," "Bulgarian B," and "Buttermilk" cultures. For differential media to demonstrate presence of pathogens in acid milk products MacConkey agar was most satisfactory; bismuth sulfite agar was also used for *Eberthella* and *Salmonella* tests, and SS agar for *Shigella* cultures. Lactic acid-producing bacteria were completely inhibited by these culture media. Titrations were made with five-ml. samples against 0.049 N NaOH, with brom thymol blue as an indicator. In order to determine total per cent acidity the formula

$$\frac{\text{ml. 0.05 N NaOH} \times 0.0045 \times 100}{\text{ml. of sample}}$$

was used. Determinations of pH values were made by the use of the Coleman pH electrometer.

Natural conditions occurring in preparation and storage of fermented milks were duplicated as much as possible in this investigation. During natural fermentation of milk, acids other than lactic are known to be present. It was decided to first compare action of pure lactic acid and mixtures of acids produced during natural fermentation. Lactic acid-producing bacteria produced different amounts of acid in sterile skimmed milk. *Eberthella typhosa, Salmonella paratyphi, Salmonella schottmülleri, Shigella dysenteriae,* and *Shigella paradysenteriae* Flexner were destroyed more rapidly when lactic acid was added to milk than when natural fermentation occurred. Results of such experiments must, therefore, be applied with caution to commercially prepared fermented milks. Inhibition of pathogens occurred within the following ranges of acidity or pH values:

	Acidity (pct.)	pH
Eberthella typhosa	0.55-0.64	4.5-4.8
Salmonella paratyphi	0.64-0.82	4.3-4.8
Salmonella schottmülleri	0.66	4.8-4.9
Shigella dysenteriae	0.56-0.67	4.8-5.0
Shigella paradysenteriae Flexner	0.53-0.72	4.6-5.0

It might be possible to infect milk before or during addition of lactic acid-producing cultures or, following acid formation, when the product is bottled for commercial distribution. Experiments were performed to determine effect of time of possible infection upon pathogens. The milk was treated much the same as it would be commercially, except for artificial inoculation of pathogenic bacteria; that is, after addition of fermenting organisms, milk samples were incubated at 37°C.(98.6°F.) until coagulated and then stored in a refrigerator. Greater longevity of pathogens was noted when acidity increased gradually than when they were inoculated into milk previously fermented. Variable resistance of organisms to germicidal agents should be considered when large numbers are inoculated. Many cells may have been destroyed, but a few highly resistant individuals may have survived and even multiplied. Slower increase in acidity permitted development of acid-tolerant strains if they were present.

It is interesting to compare results from the standpoint of acidity as expressed by pH values and per cent. The duration periods of organisms from the first experiment were chosen to compare with acidities of naturally fermented milks. In the following chart Experiment 1 results indicate survival of pathogens in sterile skimmed milk plus lactic acid, stored at 37°C.; Experiment 2 shows survival periods of pathogens in naturally fermented milks at refrigerator temperature of 8°C.(46.4°F.); and Experiment 3 notes survival of pathogens in milk inoculated with lactic acid-producing bacteria and pathogens, incubated at 37°C. until coagulated and subsequently stored at refrigerator temperature of 8°C. Milk samples were inoculated with (1) Flav-o-lac Buttermilk culture, (2) *Lactobacillus acidophilus* X, (3) *Lactobacillus acidophilus* 12, (4) Flav-o-lac Acidophilus A culture, and (5) Flav-o-lac Bulgarian B culture.

Pathogen	Exp. 2 and 3 Milk fermented by	pH	Acidity (pct.)	Pathogens destroyed in days		
				Experiment 1	Experiment 2	Experiment 3
Eberthella typhosa	1	4.9-5.1	.45-0.62	7+	28	63+
	2	4.1-4.2	.99-1.10	1	1	7
	3	4.1-4.4	.84-1.10	1-2	1	21
	4	4.0-4.2	.99-1.20	1	1	14
	5	4.5-4.8	.58-0.68	1-7	28	63+
Salmonella paratyphi	1	4.8	.57-0.62	4	58+	63
	2	4.1-4.2	.92-1.10	1	5	5
	3	4.3-4.4	.79-0.99	2	58	14
	4	4.2	.98-1.10	1	2	7
	5	4.6-4.8	.56-0.65	2-4	28	28+
Salmonella schottmülleri	1	4.7-5.1	.59-0.73	1-7+	20	63+
	2	4.0-4.4	.77-1.30	1	14	3
	3	4.2-4.5	.74-1.10	1	14	14
	4	4.0-4.4	.81-1.20	1	14	3
	5	4.5-4.9	.57-0.75	1	59+	63+
Shigella dysenteriae	1	4.4-4.7	.59-0.71	1	30	53
	2	4.1	.97-1.10	1	7	32
	3	4.2-4.3	.84-0.96	1	13	39
	4	4.1-4.2	.93-1.00	1	7	32
	5	4.8-4.9	.50-0.60	1	30	53+
Shigella paradysenteriae Flexner	1	4.5-4.7	.59-0.66	1	33	53+
	2	4.0-4.1	.94-0.98	1	7	25
	3	4.2	.86-0.98	1	14	32
	4	4.1	.91-1.10	1	7	25
	5	4.8-4.9	.52-0.56	2-3	56+	53+

Because of inadequate descriptions of care of cultures and degrees of acidity as well as methods used to compute results, it is difficult to compare results of various investigations. By using strains of lactic acid-producing bacteria which differed in amount of acid formed, it was possible to confirm almost all data recorded in literature, with a few exceptions, in spite of apparent contradictions in interpretation of results. Survival of pathogens

could not be demonstrated when acidity was greater than 1.2 per cent. This is contradictory to observations by Fügner, Kaiser, and Hansen. Longer survival periods of pathogens noted in the present investigation were probably detected through use of more selective culture media.

Possibility of transmission of pathogenic bacteria in fermented milk products cannot be overemphasized. Milk used in prepartion of these products should be subjected to temperatures above pasteurization, preferably sterilization, to eliminate pathogens and greatly reduce numbers of proteolytic organisms. Following heat treatment, milk should be handled aseptically so contamination would not be possible. The pasteurization law which makes it mandatory that all pasteurized products be bottled in the plant where pasteurized should be rigidly enforced. This legally prohibits the small plants from bottling their supply of fermented milks procured in bulk from sources which specialize in its production. Even though these regulations and restrictions may make fermented-milk products less readily procurable to the average milk consumer, he may be sure that available supplies are free from possible contamination by pathogenic bacteria.

SUMMARY AND CONCLUSIONS

Lactic acid-producing bacteria vary in amount of acid formed in sterile skimmed milk.

Eberthella typhosa, *Salmonella paratyphi*, *Salmonella schottmülleri*, *Shigella dysenteriae*, and *Shigella paradysenteriae* Flexner are destroyed more rapidly when lactic acid is added to milk than when natural fermentation occurs. Results of such experiments must, therefore, be applied with caution to commercially prepared, fermented milks.

Pathogenic organisms persist longer when acidity increases gradually. Slower increase in acidity permits development of acid-tolerant strains if they are present.

MacConkey agar is most satisfactory for cultivation of *Eberthella*, *Salmonella*, and *Shigella* species. As a second choice bismuth sulfite agar for *Eberthella* and *Salmonella*, and SS agar for *Shigella* are preferred.

Commercial buttermilk has an acidity ranging from 0.6 to 0.95 per cent. In naturally fermented milk it is not sufficient to destroy pathogens tested within a reasonable time. In fermented milk with acidity of 0.95 to 1.15 per cent (pH 3.9 to 4.2), pathogens survive from one day to seven weeks; with acidity of 0.50 to 0.86 per cent (pH 4.3 to 5.0), pathogens persist from two to nine weeks.

Fermented milks should be made only from high-grade milk pasteurized by approved methods. They should be handled as carefully as are all perishable dairy products. This is necessitated by the fact that there is wide variation in acidity and amount of contamination which might be acquired.

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EFFECT OF TIME, TEMPERATURE, AND CULTURE MEDIUM ON TOTAL COUNT OF FINE-GROUND GELATIN IN ONE-PER CENT SOLUTION[1]

THE TECHNICAL COMMITTEE OF THE EDIBLE GELATIN MANUFACTURERS' RESEARCH SOCIETY OF AMERICA[2]

(Received for publication, May 3, 1944)

In a comparative study of total counts on nutrient agar at 37°C. (98.6°F.) after 48 hours' incubation made on one- and five-per cent solutions of fine-ground gelatin, which the Technical Committee of the Edible Gelatin Manufacturers' Research Society of America published in *Food Research* (1943, 8, 429-434), it was concluded that because of its reliability, its value in appraising the sanitary quality of edible gelatin, and its greater facility of preparation and analysis the one-per cent solution of ground gelatin should be employed in preference to the five-per cent solution in the bacteriological examination for total counts.

In the light of the changes made in *Standard Methods for the Examination of Dairy Products*, eighth edition, adopted by the American Public Health Association in 1941, it was considered desirable to inquire into the relative advantages of nutrient agar *versus* tryptone-glucose agar in the bacteriological examination of edible gelatin for total counts. Simultaneously, it was decided to compare the relative merits of incubation at 28-30°C.(82.4-86°F.) *versus* 35-37°C.(95-98.6°F.) and 48 hours *versus* 72 hours. The lower temperature and longer period of incubation were selected because the official method for the bacteriological examination of edible gelatin adopted by the Canadian Government called for this procedure. In addition, the A.P.H.A. *Standard Methods for the Examination of Dairy Products* adopted in 1941 accepted incubation at 30-32°C.(86-89.6°F.) as an alternate to 35-37°C. as standard procedure. Since earlier work had demonstrated the desirability of using one-per cent solutions of ground gelatin in the bacteriological examination, this study did not introduce the additional variable of using other and stronger concentrations.

EXPERIMENTAL PROCEDURE

In all, there were 11 samples of gelatin submitted for this investigation contributed by the various companies participating in this study. These gelatins were specially selected for purposes of study and do not represent the typically low-count gelatins the companies represented normally produce.

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[1] Presented before the 73rd annual meeting of the American Public Health Association in New York on October 5, 1944.

[2] The Edible Gelatin Manufacturers' Research Society of America has the following members: American Agricultural Chemical Company; Atlantic Gelatin Company; Eastman Gelatine Corporation; Kind & Knox Gelatine Company; Milligan & Higgins Corporation; and United Chemical and Organic Products Company. Dr. Murray P. Horwood, Consulting Bacteriologist for the Atlantic Gelatin Company, Woburn, Mass., served as referee in this study.

A five-pound sample of each fine-ground gelatin studied was delivered to the referee. These samples were thoroughly mixed during the normal process of manufacture, collected aseptically, and sent to the referee in sealed, sterile containers. On receipt by the referee, each sample was mixed once more in the laboratory under aseptic conditions and then distributed into clean, new smooth cardboard containers provided with a tight-fitting cap in each case. Each container received approximately one pound of the gelatin sample. The mixing in the laboratory was performed in order to make each sample as uniform as possible. Prior to filling each cardboard container the inside was thoroughly flamed in order to eliminate any possible contamination from this source. Each container was then wrapped thoroughly and mailed to the various co-operating laboratories.

The following procedure was employed in the examination of each gelatin. Immediately before examination each gelatin was mixed thoroughly by shaking at least 25 times in the cardboard containers. After that, five separate one-gram samples were weighed out aseptically in sterile, aluminum boats and introduced into separate bottles containing 99 ml. of sterile, distilled water. The gelatin was wetted thoroughly by soaking at room temperature for 10 minutes, after which the gelatin was brought into solution promptly by placing the bottles in a water bath at 45°C.(113°F.) aided by frequent shaking. Care was taken to avoid wetting the nonabsorbent cotton stoppers used in the bottles containing the gelatin solutions.

After the gelatin was brought into solution and the solution made uniform by thorough mixing, eight separate one-ml. amounts were removed with the same sterile pipette and introduced into separate, sterile Petri dishes. The last drop in the pipette was removed either by touching a dry area of the Petri dish or by blowing it into the Petri dish. Since five one-gram samples of each gelatin were examined, each gelatin required 40 inoculations. No dilutions were made. Since the great majority of gelatins available on the market today have total counts that are lower than 10,000 bacteria per gram and many gelatins are sterile or nearly so in one-gram portions, dilutions higher than 1:100 were not considered necessary.

Total counts were made on duplicate plates from each solution of gelatin on each culture medium and at each temperature of incubation. The culture media employed were prepared from the dehydrated products of the Digestive Ferments Company. Each medium was distributed in approximately 120-ml. amounts in Erlenmeyer flasks and sterilized at 15 pounds steam pressure for 20 minutes. The medium was cooled to 45°C. in a thermostatically controlled water bath prior to pouring. Approximately 10-12 ml. of culture medium was introduced into each plate. After hardening, the plates were inverted and stored in modern incubators equipped to give uniform temperatures. All counts were made with the aid of a Quebec Colony Counter and with the covers removed. The brief exposure of each plate in this way did not result in any contamination between the 48-hour and 72-hour periods of incubation. As the 28-30°C. incubators were not equipped with cooling units, incubation at this temperature had to be abandoned when the outdoor temperature during the summer went above 30°C.(86°F.). If low-temperature incubation is to be

employed, incubators will have to be provided with cooling as well as heating units in order to make all-year-round observations possible.

DISCUSSION

Although only 11 gelatins were examined in this investigation, the fact is that actually 55 one-gram samples of gelatin were examined bacteriologically in each laboratory, since five one-gram portions of gelatin from each sample were examined. As six laboratories participated in this study and each followed the same procedure, 30 one-gram portions of each sample and 330 one-gram portions of gelatin were examined in all. Thus because of the large number of gelatin portions examined, the investigation should have greater statistical significance than if only a few samples had been examined without the repetition of each analysis on five different portions of the same sample.

Samples A, B, C, D, and E were normal, low-count gelatins. Sample C showed spreaders on tryptone-glucose agar at 35-37°C. but not on nutrient agar at 35-37°C. Spreaders occurred only rarely on both media at 28-30°C. in Sample C.

Samples F and G were moderately high in total count and showed the presence of sporeformers and spreading colonies. These conditions prevailed particularly on T. G. agar at 35-37°C. although they were not absent on nutrient agar nor at 28-30°C. Accurate counts were difficult to make under these circumstances.

Samples H and I were low in total count but both samples showed a tendency to develop molds on T. G. agar at 28-30°C.

Samples J and K were also low-count gelatins. With Sample J the counts at 35-37°C. gave appreciably higher results than at 28-30°C. This sample also showed the presence of small numbers of molds at 28-30°C.

Another observation of general interest is the fact that the colonies developing on T. G. agar both at 35-37°C. and at 28-30°C. were larger and more distinct than the colonies developing from the same samples on nutrient agar at the same temperatures. The T. G. agar is a richer nutrient medium than the nutrient agar and its general use will make for higher total counts and for the ready detection of molds in gelatin.

The tables give the average bacterial counts per gram of ground gelatin resulting from the examination of five separate one-gram portions of each sample. The data are given for each co-operating laboratory and these in turn are averaged in each case. Although average minimum and maximum counts were determined in each case, the data are not included here for the sake of brevity.

The average bacterial count at 35-37°C. for Sample A (Table 1) varied as much as 100 per cent regardless of the medium employed or the period of incubation. At 28-30°C. the variation was as much as 250 to 300 per cent after 48 hours and 200 per cent after 72 hours. Minimum and maximum bacterial counts showed equally wide variations. In general, the counts on T. G. agar were somewhat higher than the counts on nutrient agar at the same temperature and after the same period of incubation. Counts at 35-37°C. were consistently higher than similar counts at 28-30°C.

TABLE 1

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample A in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	10,600	12,800	9,900	12,000
2	8,580	8,800	8,940	8,800
3	14,400	15,400	16,300	19,300
4	15,700	14,800	16,400	15,400
5	10,000	10,700	10,900	10,600
6	16,500	18,000	17,000	18,400
Average	12,600	13,600	13,200	14,100
At 28-30°C. (82.4-86°F.)				
1	4,900	6,900	9,100	8,300
2	8,320	7,720	9,100	8,940
3	12,800	12,600	15,400	16,000
4	16,300	14,200	17,200	15,100
5	8,300	9,000	8,900	9,800
6	16,300	15,800	17,400	16,600
Average	11,200	11,000	12,800	12,500

TABLE 2

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample B in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	7,100	8,600	7,500	10,000
2	3,760	3,060	3,880	3,220
3	7,000	8,100	7,500	8,300
4	7,000	7,100	7,600	7,600
5	6,200	4,400	7,000	5,100
6	5,100	5,300	5,800	5,700
Average	6,000	6,100	6,500	6,700
At 28-30°C. (82.4-86°F.)				
1	6,400	5,100	8,600	7,400
2	2,520	2,580	2,780	2,840
3	6,300	4,900	6,600	5,700
4	6,400	7,100	7,300	7,400
5	4,500	4,600	5,000	5,100
6	4,900	5,800	5,900	6,200
Average	5,200	5,000	6,000	5,800

Sample B (Table 2) showed variations similar to those observed in Sample A. The variations in the average count were in the area of 200 per cent. This was true at both temperatures of incubation and on both media. Similar wide variations, sometimes as high as 300 per cent, were observed in the minimum and maximum counts. Again, the counts at 35-37°C. were higher than at 28-30°C.

The variation in total count in Sample C (Table 3) was relatively insignificant. There was little variation between the average mean counts and the average minimum and maximum counts. There were also minor variations between the corresponding counts at 35-37°C. and at 28-30°C.

Sample D was a low-count gelatin which showed marked variations in total count. Similar wide variations (Table 4) were observed among the average minimum and maximum counts. There was not much variation in the counts at the two temperatures but the counts on T. G. agar were appreciably higher than those on nutrient agar. The 72-hour counts were also significantly higher than the corresponding counts after 48 hours.

Sample E was a low-count gelatin with a variation in average mean count ranging from 200 to 300 per cent (Table 5). Most of the counts were remarkably uniform. The average maximum counts were only twice as high as the average minimum counts. There was not much variation between the corresponding counts on nutrient agar and T. G. agar. Counts after 72 hours were slightly higher than the corresponding counts after 48 hours.

Sample F showed variations in average mean counts between 250 and 300 per cent at 35-37°C. and 400 to 600 per cent at 28-30°C. (Table 6). Wide variations were also observed among the average minimum and maximum counts. The counts on nutrient agar were consistently higher than the corresponding counts on T. G. agar and those at 28-30°C. were materially higher than corresponding ones at 35-37°C. There was also a slight increase in total count in 72 hours over that observed in 48 hours.

Sample G showed a variation in average mean count at 35-37°C. of 250 to 300 per cent (Table 7). However, at 28-30°C. the variation ranged from 300 to 1,100 per cent. Similar wide variations were observed among the average minimum and maximum counts. The corresponding counts on nutrient agar and T. G. agar were remarkably uniform, but the counts at 28-30°C. were somewhat higher than at 35-37°C. The longer period of incubation also resulted in a slight increase in total count over that obtained in 48 hours.

Sample H was a low-count gelatin and gave remarkably uniform average mean counts (Table 8). The average minimum and maximum counts did not vary greatly from the average mean counts. The corresponding counts on T. G. agar were higher than on nutrient agar. Except for one instance, the counts on nutrient agar at 35-37°C. were slightly lower than the corresponding counts at 28-30°C. The reverse was true with T. G. agar. Again the longer period of incubation yielded somewhat higher total counts than the shorter period.

Sample I was also a low-count gelatin; the average mean counts did not show excessive variation and the average minimum and maximum counts did not deviate markedly from the average mean counts (Table 9).

TABLE 3

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample C in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	2,900	3,100	3,100	3,100
2	2,100	2,900	2,450	3,200
3	3,400	2,500	3,900	3,500
4	2,900	2,900	3,100	3,100
5	2,800	2,700	3,200	3,100
6	2,800	2,600	3,000	2,800
Average	2,800	2,800	3,100	3,100
At 28-30°C. (82.4-86°F.)				
1	2,500	3,200	2,600	3,400
2	1,690	2,660	2,130	2,810
3	2,800	3,200	3,500	4,000
4	2,300	2,900	2,600	3,100
5	1,800	2,500	2,100	3,000
6	2,300	2,800	2,600	3,200
Average	2,200	2,900	2,600	3,300

TABLE 4

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample D in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	180	550	270	780
2	70	170	90	240
3	1,200	2,700	2,500	3,600
4	100	130	120	180
5	170	190	210	240
6	760	2,800	790	2,800
Average	410	1,100	660	1,300
At 28-30°C. (82.4-86°F.)				
1	260	300	510	350
2	70	80	120	150
3	1,800	2,000	3,600	4,000
4	110	200	120	220
5	100	300	150	370
6	1,100	2,700	1,200	3,100
Average	570	930	950	1,400

TABLE 5

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample E in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	1,060	830	1,070	920
2	730	810	940	950
3	1,300	2,300	1,600	3,400
4	1,090	880	1,190	1,000
5	1,500	800	1,700	1,000
6	1,300	1,500	1,900	1,900
Average	1,200	1,200	1,400	1,500
At 28-30°C. (82.4-86°F.)				
1	680	940	770	920
2	640	860	710	1,040
3	1,400	1,700	1,800	2,700
4	1,000	770	1,170	840
5	850	700	1,100	2,100
6	690	900	1,330	1,640
Average	880	980	1,100	1,500

TABLE 6

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample F in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	9,200	9,000	10,000	8,500
2	16,860	20,520	17,500	19,660
3	12,000	11,300	15,500	15,400
4	22,800	20,500	23,000	21,000
5	12,500	6,300	11,600	7,100
6	24,900	17,100	25,500	13,000
Average	16,400	14,100	17,200	15,000
At 28-30°C. (82.4-86°F.)				
1	18,200	19,400	14,200	13,000
2	37,320	31,140	41,340	33,020
3	20,500	21,800	22,900	26,700
4	28,000	26,400	30,000	29,300
5	9,300	5,400	10,200	6,200
6	21,400	15,600	22,000	16,600
Average	22,500	20,000	23,400	20,800

TABLE 7

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample G in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	7,300	10,600	7,900	9,500
2	16,400	16,740	14,360	17,160
3	24,800	24,000	30,000	27,000
4	20,800	17,000	21,500	18,400
5	15,600	15,400	17,100	17,100
6	25,400	20,700	34,900	25,700
Average	18,400	17,400	21,000	19,100
At 28-30°C. (82.4-86°F.)				
1	5,300	9,800	6,400	10,000
2	15,980	15,860	18,680	18,680
3	26,500	26,500	32,000	30,000
4	18,400	16,800	20,000	17,600
5	14,000	13,800	15,000	14,700
6	57,200	39,100	62,100	46,100
Average	22,900	20,300	25,700	22,800

TABLE 8

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample H in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	350	1,250	340	1,500
2	30	70	40	150
3	120	190	150	190
4	100	130	130	180
5	600	490	650	540
6	160	80	220	170
Average	225	370	255	455
At 28-30°C. (82.4-86°F.)				
1	480	670	790	800
2	30	80	90	150
3	120	50	130	210
4	60	70	80	80
5	175	365	215	430
6	140	160	250	270
Average	170	230	260	325

TABLE 9

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample I in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	850	680	870	630
2	330	170	390	360
3	240	280	320	330
4	490	270	540	310
5	460	375	510	430
6	280	70	510	160
Average	440	310	525	370
At 28-30°C. (82.4-86°F.)				
1	500	580	870	840
2	10	70	60	120
3	70	250	170	290
4	190	210	420	310
5	250	235	295	290
6	160	70	170	150
Average	200	235	330	330

TABLE 10

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample J in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	1,000	1,200	1,200	1,300
2	1,460	1,760	1,640	2,140
3	1,100	1,200	1,900	1,800
4	1,500	1,500	1,640	1,700
5	135	115	200	140
6	1,700	2,450	1,850	2,570
Average	1,150	1,370	1,400	1,600
At 28-30°C. (82.4-86°F.)				
1	1,100	540	1,500	900
2	30	10	40	50
3	110	190	1,200	1,600
4	610	200	1,900	1,500
5	55	65	75	80
6	100	130	1,900	600
Average	335	190	1,100	790

Considerable uniformity of results is apparently more likely when the gelatin has a low bacterial content. The counts at 35-37°C. were consistently higher than the corresponding counts at 28-30°C. The counts on nutrient agar were higher at 35-37°C. than the corresponding counts on T. G. agar. The reverse was true, however, at 28-30°C. Again the longer period of incubation yielded somewhat higher total counts than the shorter period.

Sample J was a low-count gelatin and, except for one instance, the average mean counts were remarkably uniform (Table 10). There was also excellent agreement between the average minimum counts. The same held true for the average maximum counts. The range in counts between the

TABLE 11

Average Bacterial Counts Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions of Sample K in One-Per Cent Solution, Showing Effect of Time, Temperature, and Culture Medium

Laboratory	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
1	150	260	160	320
2	50	40	140	80
3	130	80	270	140
4	130	150	110	170
5	300	250	355	300
6	140	80	200	230
Average	150	145	605	605
At 28-30°C. (82.4-86°F.)				
1	70	400	100	870
2	20	20	70	40
3	80	110	140	130
4	110	50	160	90
5	180	130	210	155
6	80	10	180	80
Average	90	120	145	230

minimum and maximum was also not excessive, especially if the exception is excluded. Counts at 35-37°C. were materially higher than the corresponding counts at 28-30°C. At the higher temperature, the counts on T. G. agar were higher than those on nutrient agar; but at the lower temperature, the reverse was true. The longer period of incubation yielded higher total counts than those obtained after 48 hours. This was especially true at 28-30°C.

Sample K was a low-count gelatin and the average mean counts were remarkably uniform once more (Table 11). The same was true in general for the average minimum and maximum counts. While the counts varied at the end of 48 hours' incubation from 0 to 1,350, the modal count was approximately 150. There was not any significant variation in the average mean count on the two media at 35-37°C.; but at 28-30°C. the count on

T. G. agar was higher than on nutrient agar. Counts on nutrient agar were somewhat higher at 35-37°C. than the corresponding counts at 28-30°C. On T. G. agar, however, the counts at 28-30°C. were either equal to or slightly higher than the corresponding counts at 35-37°C. Again the longer period of incubation yielded higher counts than the 48-hour incubation period.

Average bacterial counts obtained by the six co-operating laboratories on each medium for each temperature and period of incubation for each of the 11 samples studied are summarized (Table 12). Thus presented, comparisons may be made more readily.

In the 11 samples of gelatin studied, eight showed counts on tryptone-glucose agar at 35-37°C. which were equal to or higher than the corresponding counts on nutrient agar (Table 13). The same result was obtained after 72 hours' incubation as well as after 48 hours' incubation.

At 28-30°C. only six of the 11 samples showed counts on tryptone-glucose agar, which were equal to or higher than the corresponding counts on nutrient agar.

The results (Table 14) indicate that after 48 hours' incubation cultivation at 35-37°C. showed equal or higher counts on nutrient agar and on tryptone-glucose agar in nine of the 11 samples studied when compared with the corresponding counts on the same media cultivated at 28-30°C. After 72 hours' incubation this disparity is somewhat diminished but is still in favor of cultivation at 35-37°C. if higher counts are desired.

DISCUSSION

The preceding analysis demonstrates that greater uniformity of observations for total counts can be obtained especially on low-count gelatins, but that variations of 200 to 500 per cent in such counts may normally be expected.

Concerning the culture medium, the evidence favors the use of tryptone-glucose extract over nutrient agar. The former agar medium usually yields higher total counts and the colonies are larger and more distinct.

Of the two temperatures of incubation employed in this study, the evidence points to the desirability of using 35-37°C. in preference to 28-30°C. The higher temperature yields a greater total count and, combined with tryptone-glucose extract agar, it yields maximum growth with clearness of colonies in the shortest possible time. The use of 35-37°C. as the temperature of incubation will also eliminate the necessity of multiple incubators in the laboratory for bacteriological supervision. It will also obviate the necessity of having cooling units in the incubator as well as heating units, especially during the summer months when atmospheric temperatures may rise to 30°C. or more for prolonged periods of time.

While an incubation period of 72 hours yields a higher total count than an incubation period of 48 hours, the difference at 35-37°C. particularly is not appreciable. Furthermore, the higher total count obtained after 72 hours does not place the gelatin qualitatively in a different class from that which the 48-hour result demonstrates. From the standpoint of supervision and regulation, the shorter period of incubation is also definitely preferable. There is no doubt that a period of incubation longer than 72 hours

TABLE 12

Average Bacterial Counts for Six Laboratories Per Gram of Ground Gelatin Resulting From Examination of Five Separate One-Gram Portions, Showing Effect of Time, Temperature, and Culture Medium

Sample	After 48 hours		After 72 hours	
	Nutrient Agar	T. G. Agar	Nutrient Agar	T. G. Agar
At 35-37°C. (95-98.6°F.)				
A	12,600	13,600	13,200	14,100
B	6,000	6,100	6,500	6,700
C	2,800	2,800	3,100	3,100
D	410	1,100	660	1,300
E	1,200	1,200	1,400	1,500
F	16,400	14,100	17,200	15,000
G	18,400	17,400	21,000	19,100
H	225	370	255	455
I	440	310	525	370
J	1,150	1,370	1,400	1,600
K	150	145	605	605
At 28-30°C. (82.4-86°F.)				
A	11,200	11,000	12,800	12,500
B	5,200	5,000	6,000	5,800
C	2,200	2,500	2,600	3,300
D	570	930	950	1,400
E	880	980	1,100	1,500
F	22,500	20,000	23,400	20,800
G	22,900	20,300	25,700	22,800
H	170	230	260	325
I	200	235	330	330
J	335	190	1,100	790
K	90	120	145	230

TABLE 13

Frequency Distribution of Average Counts for Six Laboratories on Tryptone-Glucose Agar Versus Nutrient Agar

	At 35-37°C. (95-98.6°F.)		At 28-30°C. (82.4-86°F.)	
	After 48 hr.	After 72 hr.	After 48 hr.	After 72 hr.
Number of times in 11 when average counts on T. G. agar were equal to or greater than corresponding counts on nutrient agar	8	8	6	6

TABLE 14

Frequency Distribution of Average Counts for Six Laboratories, Related to Temperature of Incubation

Variation by medium	After 48 hr.	After 72 hr.
Number of times in 11 when average counts on nutrient agar at 35-37°C. were equal to or higher than corresponding counts at 28-30°C	9	8
Number of times in 11 when average counts on T. G. agar at 35-37°C. were equal to or higher than corresponding counts at 28-30°C	9	7

would yield even higher total counts. It is necessary to select a period of incubation which reflects the quality of the product examined and which is effective for purposes of supervision and regulation. In water bacteriology, counts at 37°C. are made after 24 hours' incubation. In dairy bacteriology, all counts are made after 48 hours' incubation. Similarly, in the bacteriological examination of gelatin, very satisfactory results are obtained after 48 hours' incubation and a longer period is unnecessary.

CONCLUSIONS

Ground samples of gelatin should be thoroughly mixed prior to the bacteriological examination, but variations in total count of 200 to 500 per cent may be expected as normal.

Low-count gelatins are more likely to give uniform bacteriological results in various laboratories.

The standard medium used in the bacteriological examination of edible gelatin for total counts should be tryptone-glucose extract agar instead of nutrient agar.

The standard temperature employed in the bacteriological examination of gelatin for total counts should be 35-37°C.

The standard period of incubation employed in the bacteriological examination of gelatin for total counts should be 48 hours.

The concentration of gelatin for bacteriological analysis recommended here applies only to fine-ground gelatins. For coarse-ground and flake gelatins, higher concentrations may be necessary. This study did not inquire into the effect of this variable.

FREEZING OF APPLE JUICE

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The popularity of fruit juices has increased during recent years. The American Fruit Grower (1941) cited production of apple juice in Canada as three million gallons in 1940, compared with four hundred thousand gallons in the previous year. Mottern, Nold, and Willaman (1941) reported a probable total of five to six million gallons of apple juice in the United States in 1940. According to Pederson, Beattie, and Beavens (1941) and Beattie and Pederson (1942), blends of fruit juice, such as apple and raspberry, were believed to have great commercial possibilities. Coe (1940) suggested the use of Utah apples and cherries, unsuited for disposal as No. 1 fruit, in the form of juices.

In order to extend the marketing season, recently pressed apple juice is sometimes frozen in gallon jugs or in larger containers and later thawed and sold on the market as fresh juice. A second and a third freezing may be possible, and the problem of survival of numbers and types of organisms at various stages should be considered. Although results of microbiological tests in ampoules have been described, data relative to the effects of freezing in commercial-sized containers, such as gallon jugs, are lacking.

The object of this investigation was to study effects of alternately freezing and thawing and varying the time of thawing upon pH, total titratable acidity, refractometer reading, and upon the numbers and types of microflora in various apple juices frozen in gallon jars. Juices constantly frozen in the same type of containers for an extended period of time were used for comparison.

REVIEW OF LITERATURE

It has long been known that by virture of their organic acid content, fruits and fruit juices have bactericidal properties against certain microorganisms. Juice of half-ripe fruit, according to Ko (1918), has greater bactericidal power than ripe fruit and tannic acid is more bactericidal than citric, tartaric, or malic. Bach (1932) maintained that the undissociated part of organic acids is the active factor in germicidal action where changing pH has little effect. According to Berry (1933), destruction of microorganisms in frozen blackberries packed in cans was 99 per cent at the end of six months. The destruction of microorganisms was less rapid at —10°C.(14°F.) than at —2°C.(28.4°F.) but was considerably greater than at —20°C.(—4°F.). Similar results were obtained for strawberries and raspberries. Tetsumoto (1934) stated that the sterilizing power of fruit juices is due to action of acids, such as citric, l-malic, and d-tartaric. Wallace and Tanner (1934, 1935) found viable organisms in cans of frozen

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fruits and vegetables at the end of more than three years' freezing. Microorganism count decreased, especially during the first year. Smart (1937, 1938a,b) observed that the microorganism load in frozen blueberries decreased from an initial number varying from 100,000 to over a million per gram, to less than one per cent of the original load at the end of seven months; nevertheless, organisms remaining at the end of the investigation were sufficient to cause spoilage at room temperature. Freezing storage of blueberries for nine months at —17.8°C.(0°F.) resulted in 59.7 per cent reduction in microbial content, whereas storage at —6.7°C.(20°F.) for the same period resulted in 99.9 per cent reduction. Blueberries immersed in sirup for 24 hours at 7.2°C.(45°F.) before freezing had a higher microbial content after freezing than those frozen immediately after packing. Microorganisms most frequently isolated were common soil types without health significance but which would cause spoilage if the food was not used promptly after defrosting.

The preserving action in a combination of acetic and lactic acids in the presence of varying amounts of sucrose, according to Fabian and Wadsworth (1939), was proportional to the amount of acetic acid used. The influence of increasing concentrations of sucrose on viability of yeasts was not so great in a combination of the two acids as with acetic acid alone but greater than with lactic acid alone. Levine and Fellers (1940) observed the inhibiting effect of acetic acid upon microorganisms in the presence of sodium chloride and sucrose at equivalent pH values to be greater in tubes containing more total acid. Salt and sugar did not increase the bactericidal action of acetic acid except by altering the hydrogen-ion concentration. McFarlane (1940a, b) found the freezing patterns of water mixtures containing various concentrations of sodium chloride or sucrose at —10 to —20°C.(14 to —4°F.) to differ, as observed in the degree of concentration and modification of distribution of India ink particles and organisms. Sucrose in dilute hydrochloric acid solution altered the hydrogen-ion concentration, which affected destruction and distribution patterns of yeast cells in frozen media; viable microorganisms decreased most rapidly in samples high in soluble solids. In samples held at —10°C.(14°F.) for 31 weeks, the greatest number of viable cells was found in unfrozen concentrate, which had approximately six times the total solid and acid content of normal apple juice. In unsectioned, 750-milliliter, "malt quart" samples, greater decreases in viable microorganisms occurred in apple juice held at —20°C.(—4°F.) than in juice held at —10°C.(14°F.).

Erickson and Fabian (1942) found the decreasing order of germicidal value of certain organic acids to be as follows: lactic, acetic, and citric, if the number of grams of acid in 10 milliliters of broth was used as the basis of comparison; however, if pH was used as the basis, the order was acetic, citric, and lactic. The preserving action of sugars from microbial spoilage was in decreasing order: fructose, dextrose, sucrose, and lactose; from 15 to 17.5 per cent fructose was required to exert preserving action compared with 25 to 32.5 per cent dextrose and 40 to 60 per cent sucrose. Lactose had no preserving action. In combination with acids, fructose and dextrose were more effective than either sucrose or lactose. These investigators maintain that in an acid-sugar combination the acid is

the more important factor in germicidal action. According to Fabian and Bloom (1942), unclarified apple juice contained 40 to 50 times more microorganisms than clarified juice. Unclarified juice was unsalable as fresh apple juice after approximately seven days because of alcoholic fermentation; clarified juice was unsalable owing to acetic acid fermentation at the end of the same period. Benzoic acid inhibited the growth of microorganisms to a greater extent than monochloroacetic acid. The decreasing order of preserving action of the chloroacetic acids was mono-, di-, and trichloroacetic acids.

Forgacs (1942) found apple juice filtered through diatomaceous earth to contain 1,450 organisms per cubic centimeter as compared with 1,957,000 per cubic centimeter in unfiltered juice. According to McFarlane (1941, 1942) the number of viable *Escherichia coli* cells and *Saccharomyces sp.* did not vary with various concentrations of sucrose except when the hydrogen-ion concentration was high (pH 3.6 to 3.7). Greater destruction of *Escherichia coli* cells occurred in distilled water than in sucrose solutions, and higher concentrations of sucrose (30 to 50 per cent) tended to retard destruction. But when hydrogen-ion concentration was the only variable, destruction of *Escherichia coli* cells was greatest in sucrose solutions having greater hydrogen-ion concentrations than pH 6.5, and when the temperature was the only variable, greater decrease after several weeks' storage tended to occur at —10° C. (14° F.) than at —20° C. (—4° F.). Sucrose showed no advantage in freezing preservation of fruit juices.

The bacterial flora of mackerel decreased more rapidly at storage temperatures of —28°C.(—18.4°F.) than at —20°C.(—4°F.) for 48 hours, according to Kiser and Beckwith (1942). An approximate 100-per cent decrease occurred in suspensions of *Achromobacter* species frozen and stored at —20°C. for 15 days; a strain of *Micrococci* withstood the temperature much better. Jensen (1943) reported that supercooled water may exist in quickly chilled beef or when drops of water are quickly chilled to —12°C.(10.4°F.) or lower, which may be the state of water when molds and bacteria are observed to grow on meat at —8 to —12°C.(17.6 to 10.4°F.). He maintains that since ice crystals grow as water freezes, a layer of water more impure than the original surrounds the crystals; thus each is enclosed in a matrix having a lower melting point than itself. Since melting begins at the boundaries between crystals, microorganisms may thus be redistributed in ice. In natural ice, he maintains, bacteria are destroyed more rapidly at the temperature of the ice which is 0 to —5°C.(32 to 23°F.) than at lower temperatures; at lower temperatures bacteria remain viable for many months.

Acids having a hydroxyl group substituted for one of the hydrogen atoms, on the alpha carbon, are more bactericidal than those with three hydrogen atoms, according to Shillinglaw and Levine (1943). This might be expected from the general rule that substitution of a negative group for a positive group on the alpha carbon increases the acidity of the carboxyl. Glycolic acid was more effective than acetic under the conditions of the experiment. Death rates of organisms in acid solutions were much slower at the lower temperatures; and quantitatively the temperature effect varied with different acids. At 30°C.(86°F.) the order of increasing germi-

cidal efficiency was tartaric, phosphoric, lactic, and citric; at 6°C.(42.8°F.) the order was lactic, phosphoric, tartaric, and citric. They found the death rate of *Escherichia coli* at 30°C. to be constant throughout the course of disinfection with .02N lactic acid regardless of the size of inoculum, which appears to refute the often proposed argument that in a given sample of organisms there is a certain proportion of highly resistant forms remaining alive, or it indicates that such resistant forms are very few in any sample. Germicidal efficiency of .02N lactic acid at 30°C. was increased from 20 to 25 per cent by addition of 2.5 volumes of carbon dioxide or 10 per cent sucrose which they attributed to associative action probably affecting the permeability of the bacterial cell.

EXPERIMENTAL PROCEDURE

Apple juices for this investigation were made from Golden Delicious, Grimes, Jonathan, Willowtwig, Winesap, and Delicious apples by the following process. The apples were macerated by means of an apple grater, collected in cloths between wooden racks, and pressed in a hydraulic press. The juice was strained through cheesecloth to remove large particles and clarified by treating it with a special form of diatomaceous earth (Hyflo Super-Cel or Dicalite Speedex), which served as a filter aid, and passing it through a power filter. The juice emerged as a clear, polished product. It was run into clean gallon jugs, labeled, loosely stoppered, and divided into three lots. After samples had been removed for chemical and microbiological analyses, the juice was placed in cold storage, quickly frozen, and maintained at approximately —12°C.(10°F.). Series 3 was frozen for seven months at —23.3° C.(—10° F.), and at —17.8° C.(0° F.) for 12 months. The procedure up to the time the juice was put in cold storage was completed within 24 hours and the microorganism count was made before the juice was placed in cold storage. In addition, pH, total titratable acidity, and refractometer reading were determined before freezing.

In order to determine effects of varying the time of thawing and of the time elapsing between alternate freezing and thawing, the three lots of juice were treated as follows: Series 1 was removed from cold storage every seven days and allowed to thaw at 25°C.(77°F.) for 24 hours and refrozen. Series 2 was removed every 14 days and allowed to thaw for 12 hours and refrozen. To hasten thawing in this series, the stoppered jugs were occasionally shaken. Series 3, which had been constantly frozen, was allowed to thaw 17 hours at room temperature at the end of the experiment. All juices were analyzed microbiologically, and at the time the experiments were discontinued a chemical analysis was made of the juices of Series 1 and 2. In addition, the heights to which the jugs were filled was varied in Series 1 and 2 in order to observe possible differences owing to this factor.

Microbiological analysis was made as follows: The gallon jugs containing the juices were thoroughly shaken and the tops were wiped with a cloth saturated with 95 per cent alcohol. The mouths were then flamed. The cork of the container to be sampled was removed with a metal rod previously flamed, the mouth of the container was again flamed, and approximately 300 milliliters of juice were removed aseptically into sterile

Erlenmeyer flasks. The jugs were again stoppered and refrozen, and the juice removed was analyzed quantitatively on Petri dishes using dextrose agar as the medium. Frost's Little plates were used to a limited extent. Plates containing the medium and juice were incubated at 37°C. (98.6°F.) for 24, 48, and 72 hours, and counts were made at the end of each incubation period. Plates of Series 1 were further incubated at icebox temperatures approximating 14 to 16°C. (57.2 to 60.8°F.) for an additional five days and again counted to determine the presence of organisms growing best at such temperatures. This step was used in the first two sampling periods, but since results obtained did not show appreciable increases in microorganism number, this procedure was discontinued. The method of analysis using Frost's Little plates did not prove suitable for all samples, since the microbiological load of juice from some samples was too low to give results comparable to those obtained by the Petri plate method; it was therefore not used after the first trial. Microbiological results appear (Tables 1, 3, and 5); results showing the chemical analyses of juices before and after freezing appear (Tables 2 and 4).

Juices used varied in pH, total titratable acidity, and refractometer reading. A Beckman pH meter with a glass electrode was used to determine pH. Total titratable acidity was determined by pipetting 10 milliliters of juice into a 300-milliliter Erlenmeyer flask, adding 100 milliliters of boiling redistilled water, and titrating with standard sodium hydroxide solution of approximately 0.1 normality, using phenolphthalein as the indicator. Boiling redistilled water was used to reduce errors owing to impurities and carbon dioxide. A Zeiss hand refractometer was used to obtain data on soluble solids. Figures for refractometer reading (Tables 2 and 4) represent, roughly, the per cent of sugar in the various juices.

Juices were obtained from No. 1 apples and from defective apples which had been previously sorted on the basis of scab lesions, worm entrances, stings, size and color, and both size and color. Such juices were used in this investigation because, in previous work, noticeable differences had been observed in chemical composition; hence such juices, owing to their different pH values and total acid and sugar content, might exert different effects upon microflora.

Results from visual and organoleptic tests (taste and smell) of apple juices from Series 1 allowed to remain at room temperature (approximately 77°F.) for 24 hours are shown (Table 6). These juices were from sample bottles collected towards the latter part of the experiment to serve as an indicator of microbiological activity as well as to determine the possibility of using juices thus treated for human consumption on the basis of taste, smell, and appearance.

The microorganism count and types of organisms prevailing in juices of Series 3 at the time the experiment was discontinued appear (Table 5). Results showing types of organisms remaining in juices in Series 1 and 2 at the discontinuation of the investigation are presented (Table 7) and order of dominance of types of organisms (Table 8). The approximate microbiological condition of apple juice collected from the hydraulic press and incubated 48 hours on dextrose agar at 37°C. is illustrated (Fig. 1).

TABLE 1

Effects of Freezing and Thawing, Series 1

Variety and kind	Fill of jug	Date of sampling					
		4/16	4/23	4/30	5/7	5/14	5/21/43
G. Del.,[1] wormy	⅛	8,125	1,344T	.....[3]	.....[3]	.....[3]	.....[3]
G. Del., stung[2]	⅛	16,250	39T	432T	348T	300T	200T
G. Del., small	⅛	8,125	55T	.....[3]	.....[3]	.....[3]	.....[3]
Grimes, large, green	1/16	8,125	1T	.....[3]	.....[3]	.....[3]	.....[3]
Grimes, small, green	½	7,000	2T	350	60	20	70
Grimes (No. 1)	⅛	32,490	7,200	1,980	600	540	402
Grimes, rotten	¼	24,300	5,220	200	160	360	390
Jonathan, stung	⅛	24,200	7,200	400	140	187	160
Jonathan, wormy	½	16,251	161	.....[3]	.....[3]	.....[3]	.....[3]
Jonathan (No. 1)	⅝	16,000	100	10	23	12	20
Willowtwig, wormy	⅛	56,800	540	.....[3]	.....[3]	.....[3]	.....[3]
Willowtwig (No. 1)	½	48,700	1,500	600	380	60	54
Willowtwig, scab	½	24,370	1,920	480	210	200	275
Willowtwig, green	½	56,800	2,110	110	1	3	2
Winesap, green	½	29,384	1,120	2	1	2	0
Winesap (No. 1)	¾	24,370	500	40	420	360	6
Winesap, stung	⅛	24,300	290	400	390	10	48
Delicious, scab	⅛	24,350	174T	216T	112T	420T	700T
Delicious (No. 1)	½	77,000	143T	202T	160T	500T	800T
Delicious, wormy	½	32,500	96T	420T	264T	390T	497T
Delicious, small, red	½	40,625	117T	1,296T	.....[3]	.....[3]	3M
Delicious, stung	½	24,300	120T	225T	231T	.....[3]	198T

[1] G. Del.—Golden Delicious. [2] Stung—superficial codling moth injury. [3] Not determined.

TABLE 2

Chemical Analyses of Juices Before and After Freezing

Sample No.	Variety and kind	Acid (pct.)		pH		Refr. read.[1]	
		Before	After	Before	After	Before	After
1	G. Del., wormy	.319	.442	3.79	3.63	14.9	13.9
2	G. Del., stung	.288	.432	3.78	3.61	14.0	13.8
3	G. Del., small	.253	.377	3.94	3.93	12.6	12.2
4	Grimes, large, green	.322	.476	3.57	3.41	12.3	11.4
5	Grimes, small, green	.301	.447	3.66	3.63	11.7	11.2
6	Grimes (No. 1)	.311	.460	3.55	3.53	12.4	12.1
7	Grimes, rotten	.354	.524	3.46	.....[2]	13.4	.....[2]
10	Jonathan, stung	.497	.741	3.51	3.18	13.4	13.1
11	Jonathan, wormy	.504	.761	3.52	3.38	13.7	.....[2]
12	Jonathan (No. 1)	.484	.719	3.49	3.31	13.2	12.8
14	Willowtwig, wormy	.593	.886	3.35	3.25	13.0	12.4
13	Willowtwig (No. 1)	.506	.970	3.28	3.20	13.0	12.3
15	Willowtwig, scab	.462	.689	3.48	3.22	12.7	12.2
16	Willowtwig, green	.632	.943	3.37	3.27	10.9	10.3
17	Winesap, green	.504	.759	3.57	3.48	13.0	11.5
18	Winesap (No. 1)	.418	.627	3.65	3.42	12.4	11.8
19	Winesap, stung	.435	.652	3.61	3.52	12.1	11.8
20	Delicious, scab	.173	.257	4.09	3.89	12.4	12.1
21	Delicious (No. 1)	.180	.276	4.04	3.55	12.4	11.7
22	Delicious, wormy	.149	.204	4.06	3.51	13.9	12.4
23	Delicious, small, red	.137	.751	4.27	3.79	12.7	11.6
24	Delicious, stung	.162	.242	4.24	3.70	12.1	11.7

[1] Refr. read.—Refractometer reading. [2] Not determined.

TABLE 3

Effect of Freezing on Apple Juice, Series 2

Sample No.	Variety and kind	Fill of jug	Date of sampling		
			4/14	4/29	5/14
2	G. Del., stung	¾	65,000	14,400	7,800
3	G. Del., small	¾	57,000	6,100	1,700
4	Grimes, large, green	¾	48,000	1,330	750
5	Grimes, small, green	¼	32,000	400	260
6	Grimes (No. 1)	¾	16,000	1,700	1,000
9	Jonathan, stung	¾	24,000	100	20
10	Jonathan, wormy	¾	16,000	0	2
11	Jonathan (No. 1)	¾	24,000	1,900	110
12	Willowtwig (No. 1)	¾	32,000	20	Spr.[1]
13	Willowtwig, wormy	½	24,000	70	Spr.
14	Willowtwig, scab	½	16,000	140	10
15	Willowtwig, green	¾	32,000	200	12
16	Winesap, scab	¾	16,000	100	0
17	Winesap, green	⅛	32,000	6	1
18	Winesap, (No. 1)	¾	32,000	10	0
19	Winesap, stung	¾	16,000	20	0
20	Delicious, scab	¾	24,000	15,500	6,600
21	Delicious (No. 1)	¾	24,000	11,400	Spr.
22	Delicious, wormy	¾	51,000	36,700	12,200
23	Delicious, small, red	⅛	500,000	192,000	78,000
24	Delicious, small, stung	¾	16,000	14,200	7,800

[1] Spr.—spreader; such samples could not be counted.

TABLE 4

Chemical Analyses of Juices Before and After Freezing

Variety and kind	Acid (pct.)		pH		Refr. read.	
	Before	After	Before	After	Before	After
G. Del., stung	.288	.421	3.78	3.61	14.0	13.8
G. Del., small	.253	.367	3.94	3.90	12.6	12.2
Grimes, large, green	.322	.470	3.57	3.56	12.3	12.0
Grimes, small, green	.301	.415	3.66	3.63	11.7	11.5
Grimes (No. 1)	.311	.450	3.55	3.53	12.4	12.1
Jonathan, stung	.497	.601	3.51	3.42	13.4	13.2
Jonathan, wormy	.504	.673	3.52	3.38	13.7	13.0
Jonathan (No. 1)	.484	.681	3.49	3.36	13.2	12.8
Willowtwig (No. 1)	.....	.604	3.28	3.35	13.0	12.3
Willowtwig, wormy	.593	.631	3.35	3.27	13.0	12.4
Willowtwig, scab	.462	.589	3.48	3.25	12.7	12.0
Willowtwig, green	.632	.803	3.37	3.28	10.9	9.7
Winesap, scab	.435	.....[1]	.....[1]	3.44	.....[1]	11.8
Unknown variety	.504	.631	3.57	3.54	13.0	12.7
Winesap (No. 1)	.418	.583	3.65	3.54	12.4	11.6
Winesap, stung	.435	.594	3.61	3.59	12.1	11.6
Delicious, scab	.173	.243	4.09	4.00	12.4	11.8
Delicious (No. 1)	.180	.281	4.04	3.81	12.4	11.1
Delicious, wormy	.149	.312	4.08	3.51	13.9	12.4
Delicious, small, red	.137	.789	4.27	4.04	12.7	11.1
Delicious, stung	.162	.234	4.24	4.17	12.1	11.7

[1] Not determined.

TABLE 5

Effects of Constant Freezing, Series 3

Variety and kind	Fill of jug	Organisms per c.c.	Types of organisms
Grimes, small, green	¾	180	Few yeasts; mostly gram-positive and gram-negative rods and cocci.[1]
G. Del., stung	¾	1,210	"
Jonathan (No. 1)	¾	126	"
Jonathan, stung	¾	211	"
Willowtwig, green	¾	123	"
Willowtwig, green	¾	116	"
Winesap, (No. 1)	¾	266	"
Winesap, green	¾	240	"
Winesap, large, stung	¾	198	"
Delicious (No. 1)	¾	1,211	"
Delicious, stung	¾	1,980	"
Delicious, stung	¾	2,940	"
Delicious, scab	¾	1,259	"
Delicious, small, red	¾	1,380	"

[1] Juices at the end of the experiment, according to organoleptic tests, were almost normal, that is, they were sweet to taste and very little "off-flavor." In addition, at the end of 48 hours' storage at 17.2°C.(63°F.) no detectable fermentation was observed.

TABLE 6

Organoleptic and Visual Tests of Juices Alternately Frozen and Thawed

Sample No.[1]	Condition
1	Fermentation, sour.
2	Fermentation, sour, pellicle formed.
3	Fermentation, sour, slight pellicle formation.
4	Fermentation, sour, heavy pellicle formation.
5	Fermentation, sour, slight pellicle formation.
6	Fermentation, sour, heavy pellicle formation.
7	Fermentation, semi-sour, no pellicle formation.
9	Fermentation slight, no pellicle formation.
10	Fermentation slight, sour, slight pellicle formation.
11	Fermentation, no pellicle formation.
12	Fermentation, sour, no pellicle formation.
13	Fermentation, sour, no pellicle formation.
14	Fermentation, no pellicle formation.
15	Fermentation, not sour, no pellicle formation.
16	Fermentation, not sour, no pellicle formation.
17	Fermentation, not sour, no pellicle formation.
18	Fermentation, slightly sour, no pellicle formation.
19	Fermentation, sweet, pellicle formation.
20	Fermentation, heavy pellicle, offensive odor.
21	Fermentation, heavy pellicle, offensive odor.
22	Fermentation, heavy pellicle, "yeasty odor."
23	Fermentation, heavy pellicle, offensive odor.
24	Fermentation, heavy pellicle, offensive odor.

[1] See Tables 2 and 3.

CONCLUSIONS

Range in per cent acidity, according to Tables 2 and 4, was from 0.137 in Delicious juice, made from apples that were red but under 2½ inches in diameter, to 0.635 in juice from green Willowtwigs. In general, the order of increasing total acidity was as follows: Delicious, Golden Delicious, Grimes, Winesap, Jonathan, and Willowtwig. Hydrogen-ion concentration as indicated by pH varied from 4.27 for juice made from small, red, Delicious apples to 3.28 for juice from No. 1 Willowtigs. In general, pH varied in approximately the same order as acidity. The lowest refractometer reading, 10.9, was obtained in juice from green Willowtwigs and the highest, 14.9, in juice from Golden Delicious apples with worm entrances. There was no correlation between refractometer reading and total acid content or pH.

TABLE 7

Examination of Types of Organisms Remaining in Juices From Series 1 After Alternately Freezing and Thawing

Sample No.	Description of colonies, organisms, etc.
1	Small, yellow, round, smooth colonies; many gram-negative, small rods occurring in two's.
2	Hazy colonies; rough edges. Small, gram-positive rods, sporeformers; *Penicillium sp.*
3-5	*Dermatium sp., Aspergillus sp.*
6-7	Small, round colonies; gram-positive, long rods occurring in pairs and in three's. Few gram-negative, small rods in two's.
17	Gram-positive, long rods occurring singly; sporeformers.
18	Gram-negative, small rods occurring in two's.
19	Gram-positive, long rods; sporeformers.

TABLE 8

Order of Dominance of Types of Organisms in Series 1 and 2 at End of Investigation

1. Gram-negative, very small, nonsporeforming rods.
2. Gram-positive, long, sporeforming rods.
3. Gram-positive, short, sporeforming rods.
4. Yeasts.
5. *Penicillium sp.*
6. *Aspergillus sp.*
7. *Dermatium sp.*

Concerning the microbiological data, the initial microorganism number, in general, was approximately the same for the same varieties listed (Tables 1 and 3); however, rather wide differences existed in a few cases which cannot be accounted for with certainty. Possible contamination during handling prior to freezing could have been a cause. It is logical to assume that containers varied in microbial content, which fact would account for some difference. In addition, since the juice during filtration

was allowed to flow directly into gallon containers, the juice first to enter would very likely contain the greatest number of microorganisms.

The death rate, in general, appeared to be more rapid in juices in Series 2, which, although they were thawed every two weeks, were thawed completely in 17 hours compared with a 24-hour thawing period of juices in Series 1. Death rate was slower in Delicious and Golden Delicious juices in Series 2, and the numbers actually increased in juices of both varieties in Series 1. This is well illustrated in juice made from small, red, Delicious apples which had an initial number approximating 41,000 organisms per milliliter and a final number of three million per cubic centimeter. In general, the microbial load decreased more rapidly in juices having the

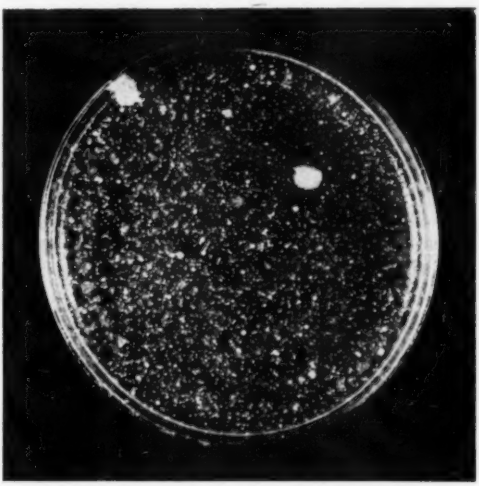

FIG. 1. Organisms from juice taken from hydraulic press. Dilution 1:100, dextrose agar medium.

greater total titratable acidity and hydrogen-ion concentration. Absolute sterility was obtained in only a few cases, although numbers decreased considerably in many cases. Although a difference existed between death rate and degree of fill of containers, the data cannot be interpreted on this basis since total acid, pH, and refractometer reading varied in each container; hence differences attributed to fill might easily be due to one or more of the variable factors mentioned.

Chemical analyses after freezing showed wide variations in per cent acid, pH, and refractometer reading which seem to be correlated with the number of microorganisms remaining at the time the investigation was discontinued. Total acid content and hydrogen-ion concentration increased and refractometer reading decreased in all cases, which may be accounted for by decrease of sugar content owing to action of microorganisms. The amount of change in pH was not constant for all juices, which is probably due to differences in buffering capacity.

With regard to the type of organisms remaining at the end of the experiment, according to results in Table 5, juice which had been frozen constantly contained mostly gram-positive and gram-negative rods, cocci, and very few yeasts. The number of microorganisms remaining in this series of juices was relatively low; however, the less-acid juices contained the higher number of microorganisms. Bacteria predominated in juices from Series 1 and 2 at the time the investigation was discontinued; yeasts and molds were few in number as is shown (Tables 7 and 8). Of the bacteria, gram-negative, small, nonsporeforming rods were most prevalent, and the gram-positive, sporeforming rods were next.

Differences in the appearance and taste of juices from Series 1 and 2 were observed (Table 6). Most of the juices were sour, some were sweet, and others were not tasted because of the presence of offensive odors or of an unsightly appearance. Signs of spoilage were present in all samples alternately frozen. Juices which were constantly frozen were normal in appearance, aroma, and taste. A final chemical analysis was not made of the latter.

The above data illustrate the importance of the rapid handling of frozen juices in order to prevent spoilage by microorganisms. By personal correspondence, information has been obtained relative to cases where apple juice had been frozen in containers as large as 10-gallon barrels, the contents of which were later dispensed from the tap as a fountain drink. This is almost inconceivable because of the microorganism load in such a container. Furthermore, organisms remaining at the end of a freezing period, according to data presented above, probably will bring about putrefaction and acid formation, rather than the formation of the alcohol and carbon dioxide which make the autumn drink called hard cider.

SUMMARY

A study was made on the effects of alternate freezing and thawing, as well as of varying the time of thawing, upon pH, total titratable acidity, and refractometer reading and upon the microflora of various apple juices frozen in gallon jugs. Juices continuously frozen for approximately two years were examined microbiologically and organoleptically.

A. In general, there was an increase in acidity and hydrogen-ion concentration in all samples.

B. The refractometer reading, which is the per cent soluble solids (a rough estimate of per cent sugar), decreased. There was no constant correlation between refractometer reading and total acid content or pH.

C. The death rate of microorganisms in general was more rapid in juices thawed for 17 hours and refrozen every two weeks than in those thawed for 24 hours and refrozen every week. In fact, in Delicious and Golden Delicious juices, there was an increase in the total number of microorganisms in juices thawed for 24 hours.

D. In juices thawed every 17 hours, the death rate was the slowest in Delicious and Golden Delicious juices which were considerably less acid than the other varieties used.

E. Absolute sterility was obtained in only a few cases, although total number of microorganisms decreased greatly in the juices which were more acid.

F. The order of dominance of microorganisms present when the investigation was discontinued was gram-positive rods, gram-negative rods, cocci, and yeasts.

G. Most juices which were thawed and refrozen were unsuitable for human consumption when the investigation was discontinued.

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A COMPARISON OF MEASUREMENT OF JUICINESS IN ROAST PORK LOIN BY PRESS-FLUID AND JURY-RATING METHODS [1]

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Juiciness is an important factor in the palatability of meat and one on which individual judgments vary greatly. An objective method for its measurement, therefore, would be valuable. The pressometer was developed for this purpose, but only a few comparisons between percentage of press fluid and the quantity of juice have been reported. In one such comparison, made on 72 pork loin roasts cooked in three different ways, Smith (1933) found in each group a correlation of less than 0.1 between the percentage of press fluid and the average of the scores of three judges on quantity of juice. In another Satorius and Child (1938a), using 39 standing rib roasts of beef and five judges, found a correlation of 0.31, which was insignificant, between the percentage of press fluid and average score for juiciness. The correlation, however, between the combined scores for flavor and aroma [2] and the combined scores for quality and quantity of juice was 0.68, which was highly significant; on the other hand, the correlation between flavor-aroma and aroma was 0.01 and nonsignificant. The authors suggest that the judges' scores on juiciness may have been affected by other palatability factors which stimulated the flow of saliva, and that this may have accounted for the high correlation of flavor-aroma and the quality-quantity of juice and the lack of correlation between press fluid and jury rating on quantity of juice.

In view of the value of the objective test, if proved applicable, and of the small amount of work reported with it, another comparison was thought to be worth while. The present paper discusses results of that comparison.

EXPERIMENTAL PROCEDURE

The data were collected on four series of pork loin roasts which formed part of the experimental material in a study of palatability changes during freezing-storage. Each series (as used in this study) consisted of 108 roasts from 18 animals of known feed history and breed.[3] The loins were cut into roasts, wrapped in moisture-vapor-proof paper, and frozen at —18° C. (0°F.) on the third day after slaughter. Immediately after freezing, all

[1] Scientific Journal Series paper No. 2171, Minnesota Agricultural Experiment Station.

[2] Combined scores were used because the correlations between the factors involved were high and significant; for example, the correlation between flavor and aroma was 0.7, and between judges' scores for quality and for quantity of juice, 0.8.

[3] Hampshire, Chester White, Duroc Jersey, and Poland China breeds, eight to nine months old, average weight 240 lb., fed on a ration of shelled corn, tankage (self-fed), a mineral supplement, buttermilk, and alfalfa hay.

of the roasts from one-third of the animals were stored at —18°C.(0°F.), those from another third at —12°C.(10°F.), and the remaining third at —9°C.(15°F.). At the end of the following storage periods, one roast (selected at random) from each animal was withdrawn, cooked, and analyzed: for Series 1—2, 4, 6, 8, 10, and 12 weeks; for Series 2—16, 18, 20, 22, 24, and 26 weeks; for Series 3—3, 6, 9, 12, 15, and 16 weeks; and for Series 4—18, 21, 24, 27, 30, and 33 weeks.

Since the laboratory facilities were not such that 18 roasts could be handled in one day, the roasts from half of the animals in each series were put in storage at one time and those from the other half at a somewhat later date (the interval varied from one to three weeks)—that is, although the six roasts stored at, for example, —18°C. for two weeks, were considered as a unit, actually three of them were judged at one time and the other three at least a week later. This is a minor point, but interesting in view of some of the findings of the study.

At the end of each storage period the roasts which were to be cooked at that time were removed from storage and thawed overnight in a refrigerator at approximately 7°C.(44.6°F.). The next morning they were placed fat side up on a rack in a roasting pan, a thermometer inserted so that the bulb was as nearly as possible in the center of the roast, and the roasts set in an oven at 176°C.(350°F.). When the internal temperature of the meat reached 84°C.(183°F.), the roast was taken out of the oven and allowed to come to maximum temperature. The longissimus dorsi muscle was removed, cut in half crosswise through the thermometer hole, and a one-eighth-inch slice was taken from the left half and one or two similar slices from the right half; each of these slices was cut into two pieces, which were given to the judges for tasting, each judge always receiving a sample from the same part of the roast. A slice 1.25 cm. thick was cut off the left half of the muscle adjacent to the one removed for the judges. From this slice two samples were taken, one from each side of the thermometer hole, with a cork borer 1.25 cm. in diameter. These samples were placed in weighing pans and used for press-fluid determinations, to be described later.

Four or five experienced judges scored the roasts, using the seven-point grading sheet recommended by the cooking committee of the Cooperative Meat Investigations Project—Alexander, Clark, and Howe (1933). A copy of the grading sheet may be found in papers by Satorius and Child (1938 a, b) and by Shrewsbury *et al.* (1942). In every series one judge was absent at least once; in each series, therefore, the ratio of his scores to the average scores of the other judges was determined for each judging time for which he was present, and the scores for those times that he was absent were calculated from this ratio.

The samples taken for press fluid were placed in weighed tin-foil cups one-half inch in diameter and three-fourths inch deep, which in turn were placed in weighed aluminum pans with tight-fitting covers. After the pans containing the foil and samples had been weighed, the tin-foil cup was discarded (and with it any moisture which had condensed on the foil). The samples were then wrapped in cross-shaped pieces of filter cloth which previously had been boiled 10 minutes in distilled water and dried. They

were then subjected to 250 pounds of pressure per square inch for 10 minutes, after which they were returned to the weighing pans and reweighed. The difference in the weight of the sample before and after pressing was called press fluid and was expressed in percentage of the original sample. The two measurements of each roast were averaged.

In this study, correlations of press fluid and the average score of the judges for quantity of juice were calculated; and after the correlations were found to be low, an analysis of variance and covariance was made of each series, in order to see, if possible, the cause of the low correlation.

RESULTS AND DISCUSSION

The coefficients of correlation between percentage of press fluid and jury rating of quantity of juice in the four series studied were as follows: Series 1, 0.32; Series 2, 0.38; Series 3, 0.51; and Series 4, 0.39. All of these coefficients are statistically highly significant, according to Fisher (1932), but they are so low that they are not of much practical value in predicting judges' scores from press-fluid measurements. An analysis of variance and covariance was made, therefore, for each series in order to see whether the differences in the percentage of press fluid and scores *between* tests (a test was considered to be the six roasts stored for the same period at the same temperature) were greater than the differences *within* tests (Table 1). As will be noted, there was a significant difference

TABLE 1

Analysis of Variance and Covariance of Percentage of Press Fluid and Judges' Average Scores for Juiciness in Each of Four Series of Pork Loin Roasts[1]

Series	DF	Mean square due to		
		x^2	xy	y^2
Series 1				
Between tests	17	60.3800[2]	0.2100	0.7275
Within tests (error)	90	20.4432	1.4700	0.5612
Total	107	.....	.....	.....
Series 2				
Between tests	17	39.0753	2.8005	1.1112
Within tests (error)	90	22.5239	1.4387	0.6804
Total	107	.....	.....	.....
Series 3				
Between tests	17	21.1161	1.6491	0.6027
Within tests (error)	90	20.2492	2.1499	0.8659
Total	107	.....	.....	.....
Series 4				
Between tests	17	18.2236	0.0009	0.6049
Within tests (error)	90	25.7080	1.7381	0.5634
Total	107	.....	.....	.....

[1] 108 roasts in each series; x = percentage of press fluid, y = judges' average scores. [2] Indicates significance at the one-per cent level.

in the percentage of press fluid between tests in only one series, the first; and in no series were the judges' average scores significantly different between tests. In other words, for both press fluid and judges' scores the variation within the tests was in general as great as the variation from one test to another. This finding indicates that neither time nor temperature

of storage had an effect on the quantity of press fluid or judges' scores for juiciness greater than the variation between individual roasts.

The correlation coefficients of press fluid and jury ratings between and within tests were then calculated and are shown (Table 2) together with the total correlation coefficients. The correlation between tests was non-significant in all series, but the correlation within tests was highly significant in all series. If we assume that the pressometer, being mechanical, does not vary from one test to another, we must assume that the judges maintain a certain standard of juiciness within one test, but that this standard changes from one test to another. The latter assumptions are especially interesting in view of the fact, as mentioned in the method, that inadequate laboratory facilities forced judging one-half of the roasts belonging to any one test at one time, the second half being judged one to three weeks after the first. Such maintenance of judging standards over a period of time indicates that the judges were scoring a real factor, while the seeming change of standard between tests suggests that the factor was not the quantity of juice alone, but a combination of juiciness with other factors which were influenced by the time or temperature of storage. Other data on these four series show that in general the only palatability factors which changed significantly with storage time or temperature were flavor and aroma, the same factors which were shown by Satorius and Child (1938a) to be significantly correlated with quality-quantity of Juice.

TABLE 2

Correlation Coefficients of Percentage of Press Fluid With Judges' Average Scores for Juiciness Between and Within Tests on Four Series of Pork Loin Roasts[1]

Series	Between tests 17 D.F.	Within tests 90 D.F.	Total 107 D.F.
1	.03	.43[2]	.32[2]
2	.42	.37[2]	.38[2]
3	.46	.51[2]	.51[2]
4	.00	.46[2]	.39[2]

[1] 108 roasts in each series. [2] Indicates significance at the one-per cent level.

SUMMARY AND CONCLUSIONS

A study was made of the percentage of press fluid and the average of judges' scores for quantity of juice in each of four series of pork loin roasts kept in freezing-storage at —18, —12, and —9°C. (0, 10, and 15°F.) for two to 33 weeks. After the roasts (432 in number) were cooked and sampled according to standard methods, they were scored by four to five experienced judges and the amount of press fluid was determined.

The quantity of press fluid and the judges' scores on juiciness were studied by analysis of variance and covariance. It was found that the amount of press fluid in all but one series did not vary significantly between tests (a test being the six roasts stored at a given temperature for a given time). Similarly, there was no significant variation between tests on judges' scores for juiciness. These results showed that the difference in these factors among individual roasts were as great as differences caused by the effect of time and temperature of storage.

The correlation coefficients between percentage of press fluid and judges' scores on juiciness varied from 0.32 to 0.51 for the four series. All were highly significant because of the large number of degrees of freedom (107), but were too low to make it practicable to predict judges' scores on juiciness on the basis of press-fluid determinations.

When the correlations were broken down into their component parts, that is, the correlation within tests (a given storage period and temperature) and the correlation between tests, it was found that the correlation coefficients between tests were nonsignificant. This finding indicates that the judges' scores for quantity of juice were not in themselves significantly affected by storage time or temperature but does not preclude their being related to some factor which is affected by time and temperature of storage. The correlation coefficients within tests were highly significant, although part of a test was judged at one time and the rest of it a week or more later, showing that the judges had an absolute standard of juiciness or of juiciness and the factor or factors which may be associated with it in the judging.

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EFFECT OF STORAGE TEMPERATURE AND TIME UPON QUALITY OF PORK PRESERVED BY FREEZING[1]

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The increasing use of the freezer-locker as a means of preserving meat and the questions resulting from its use have served to emphasize the meagerness of the experimental data upon which to base recommendations as to the length of time pork may be stored in family-sized pieces at freezing temperatures without serious deterioration in palatability. The present study was initiated to furnish data which would help in making such recommendations. While it was in progress, several other studies of a similar nature were reported. In one of them, DuBois, Tressler, and Fenton (1940) reported, as the result of active oxygen and organoleptic tests on the fatty tissue from the loin of one hog, that "pork becomes rancid in about four months . . . at 10 to 15°F. At 0°F. pork shows some signs of rancidity in 12 months." Neither active oxygen values nor judges' scores are given, so that comparison with other studies on either of these bases is not possible. In another study, Wellington, Mackintosh, and Vail (1940) state that their palatability committee agreed pork roasts stored at a temperature ranging from 7 to 18°F. should not have been held longer than 150 days. This decision must have been based upon some factor or factors not reported. It could not have been the result of the palatability scores on the fat, since the fat from four of the eight animals used scored lower at the end of 30 days of storage than at the end of 150 days. Recently Hiner and Kauffman (1944) have reported higher scores for the desirability of the flavor of both the fat and lean from three pork loin roasts stored at 0°F. than from the other member of the pair stored at 18°F. The storage periods were 12, 24, and 36 weeks. At both temperatures, however, the flavor of the fat was described as only "slightly desirable" at the end of 12 weeks of storage. Shrewsbury *et al.* (1942) also have made an extensive examination of the palatability changes in pork during freezing-storage, but their storage temperatures were considerably lower than any used in the present study.

EXPERIMENTAL PROCEDURE

Selection, Preparation, and Storage of Samples: Thirty-six choice butcher hogs[2] which had been raised at University farm on a ration of shelled corn, tankage (self-fed), a mineral supplement, buttermilk, and alfalfa hay were slaughtered at a local packing plant. The carcasses were chilled at —1.7 to 0°C.(29 to 32°F.) for 24 hours, then cut into wholesale pieces, and the loins immediately taken for the experiment.

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[1]Scientific Journal Series paper No. 2170, Minnesota Agricultural Experiment Station.

[2]Hampshire, Chester White, Duroc Jersey, and Poland China breeds, eight to nine months old, average weight 240 lb.

Each pair of loins was cut into eight roasts, one of which (selected at random) was cooked and tested at once, while the others were weighed, double-wrapped in moisture-vapor-proof paper, reweighed, and then frozen by being spread out in a room at —18°C.(0°F.) for 48 hours. After freezing, a second roast (also selected at random) from each lot of eight was cooked and tested. The remaining six were assigned as a unit to a storage temperature, those from one-third of the animals to —18°C.(0°F.), those from another third to —12°C.(10°F.), and those from the remaining third to —9°C.(15°F.). They were then distributed at random among the storage periods of 3, 6, 9, 12, 15, and 18 weeks or among the periods 18, 21, 24, 27, 30, and 33 weeks. (Because of limited storage space the samples, of necessity, were handled as two separate groups treated alike except for the storage periods.) The storage space available at —18°C. consisted of a dimly lighted room, which was regulated within ±0.5°C.(±1°F.) of the desired temperature, but which contained other products besides the experimental pork. The space at —12°C. and at —9°C., on the other hand, consisted of refrigerated compartments in a well-insulated cabinet. The compartments were barely large enough to hold the experimental samples and, as might be expected, were dark. Each was equipped with a recording thermometer and a thermostat which held the temperature within ±0.5°C.

Thawing and Cooking of Samples: At the end of the storage period the roasts were unwrapped, placed fat side up on a wire rack in an open pan, thawed in a refrigerator at approximately 7°C.(45°F.), cooked at 175°C. (350°F.) to an interior temperature of 84°C.(183°F.), removed from the oven, and allowed to stand until the maximum internal temperature was reached.

The fresh roasts, mentioned previously, were cooked by the same method as the thawed ones.

Storage, Thawing, and Cooking Losses: In order to have a record of the losses occurring at various stages, the roasts were weighed before and after storage (as has been stated), before and after thawing, after cooking, and after reaching the maximum internal temperature. The weights of the drippings collected during thawing and during cooking were recorded also.

Scoring of Samples: Each roast, after its last weighing, was cut through the center across the grain of the meat, and a thin slice (0.3 cm. thick) removed from either side of the cut. The slices were divided into halves, and each half scored by an experienced judge according to the seven-point scale [8] of the cooking committee of the Cooperative Meat Investigations Project—Alexander, Clark, and Howe (1933)—except that the fat was divided into two portions and each scored separately. One portion consisted of a well-defined, brown layer about three mm. thick which covered each roast; the other was the fat which lay directly beneath the outside layer. This separation was made because of the possibility that the surface fat might possess characteristics different from the interior fat, since the roasts were not trimmed after removal from storage.

The aroma was judged in each case from half of a roast and consisted of a combination of the odors given by the fat and lean.

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[8] Summarized in the first footnote to Table 1, and given in detail by Shrewsbury *et al.* (1942).

Press-Fluid and Freezing-Drip Determinations: A slice 1.3 cm. thick was carved from the left half of the roast adjacent to the slice used for judging. It was then cut into five samples, each 1.25 cm. in diameter. Two of the samples were used for press-fluid determinations, according to the method of Child and Baldelli (1934), and three for freezing-drip determinations, according to the method of Cook, Love, Vickery, and Young (1926).

RESULTS

Quality of Experimental Samples as Measured by Judges' Scores: In spite of the careful selection and prompt use of the experimental material, the fresh roasts after cooking were rated by the judges as only moderately desirable to desirable—that is, the average scores for the various factors ranged for the most part between five and six out of a possible seven points, as may be seen from the first column of figures (Table 1). The reason for the lowness of these scores is not clear. The quality of the pork could hardly have been at fault. A more likely explanation is that the judges were unusually critical, owing to the focusing of their entire attention upon the meat. Whatever the explanation, however, the scores show the advisability of judging control samples in a study dealing with possible deterioration in quality.

TABLE 1

Effect of Freezing at —18°C.(0°F.) Upon Quality of Pork Roasts

Factor [1]	Mean for all roasts which had not been frozen [2]	Mean for all roasts which had been frozen but not stored [2]
Desirability of flavor of interior fat	6.0	5.9
Desirability of flavor of exterior fat	5.2	5.1
Desirability of flavor of lean	5.9	5.8
Desirability of aroma	4.9	5.1
Intensity of flavor of interior fat	5.0	5.3
Intensity of flavor of exterior fat	5.8	6.0
Intensity of flavor of lean	5.9	6.1
Intensity of aroma	5.0	5.0
Tenderness	5.3	5.6
Richness of juice	5.6	5.5
Quantity of juice	5.2	5.2
Cooking loss, in percentage of raw weight	28.4	24.4
Total loss, in percentage of raw weight	28.4	26.6
Press fluid, in percentage of raw weight	36.4	38.3
Freezing drip, in percentage of raw weight	11.1	11.7

[1] The first 11 factors are reported in terms of the mean of the judges' scores. The maximum score possible in each case is 7. For the various desirability and intensity factors, the numerical scores are described as follows on the score sheet: 7, 6, 5, 4 very desirable or very pronounced, desirable or pronounced, moderately desirable or moderately pronounced, and slightly desirable or slightly pronounced, respectively; and 3, 2, 1 as neutral or perceptible, slightly undesirable or slightly perceptible, and undesirable or imperceptible. [2] None of the differences between the means for the roasts which were and were not frozen are significant, according to the "t" test.

Effect of Freezing: As will be noted (Table 1) freezing altered none of the characteristics of the roasts, according to the "t" test. Not even such factors as tenderness, loss in weight during thawing and cooking, the amount of freezing drip, or the quantity of juice as judged from both press-fluid and judges' scores were affected.

Effect of Storage Temperature on Palatability: In order to find the effect of the storage temperature upon the palatability of the roasts, the data were analyzed in two ways: (1) the significance of the difference between the regression coefficients of the individual palatability factors on the length of storage at the different temperatures was determined, and (2) an analysis of variance was made of the judges' scores recorded at each of the storage periods for the four palatability factors which showed greatest change. The first of these two treatments was made to find whether the rate of deterioration was the same or different at the different temperatures, and the second to find at how many of the judging periods temperature influenced the scores significantly.

The differences among the regression coefficients for the three storage temperatures were not significant for any of the palatability factors. In other words, the rate at which the individual palatability factors changed during storage was not influenced by the temperature at which the roasts were held. The data upon which this statement is based and the F values which were used to test the significance of the difference among the regression coefficients are given (Table 2) for the four quality factors which showed greatest change during storage. As will be noted from the table, none of the F values was significant.

The analyses of variance, like the regression coefficients, showed that the storage temperatures were not significantly different from one another in their effect upon the palatability of the roasts, for they caused significant variation at only a few storage periods in the scores given to the various palatability factors—at two (out of a possible 12) periods, each, in the scores for the desirability of the flavor of the interior fat and lean, at four for the exterior fat, and at one for the aroma. These periods are too few to be considered of much importance, and even the small amount of importance which might be attached to them disappears when one notes (Table 2) that the temperature did not affect the scores in a consistent way. For example, at one of the two periods (18 weeks) when storage temperature significantly influenced the flavor of the interior fat, the highest temperature (—9°C.) gave the most desirable flavor, while at the other period (30 weeks) it gave a flavor intermediate in desirability. Similar inconsistencies are found when the other palatability factors are considered.

Thus, both treatments of the data show that no one of the temperatures preserved the palatability of the roasts to a greater degree than either of the others. This finding does not agree with that of DuBois, Tressler, and Fenton (1940), who (as previously noted) report that the fat of pork chops stored at —9°C.(15°F.) was definitely rancid at the end of two months, while that of chops stored at —12 and —18°C.(10 and 0°F.) did not become so until four and 14 months, respectively, had elapsed; nor does it agree with the results of Hiner and Kauffman (1944), who report consistently higher average scores for the desirability of the flavor of the fat and lean for roasts stored at 0°F. than for ones at 18°F. The reasons for the disagreement are obscured by differences in method of obtaining and reporting the data. One of the obvious differences, however, was in the number of animals from which the samples were taken—one and three,

TABLE 2

Judges' Average Scores for Desirability of Pork Roasts Stored for Varying Lengths of Time at —18, —12, and —9°C.(0, 10, and 15°F.), Coefficient of Regression of These Scores Upon Time of Storage, and Significance of Difference Between Regression Coefficients

A. Storage Periods From 0 to 18 Weeks

Storage period (wks.)	Judges' scores for desirability of flavor of interior fat			Judges' scores for desirability of flavor of exterior fat			Judges' scores for desirability of flavor of lean			Judges' scores for desirability of aroma		
	—18°C.	—12°C.	—9°C.	—18°C.	—12°C.	—9°C.	—18°C.	—12°C.	—9°C.	—18°C.	—12°C.	—9°C.
0	6.03	6.09	6.42	5.36	5.19	5.54	6.26	5.87	6.09	5.80	5.69	5.75
3	5.66	5.67	5.09	3.94	5.32	4.58	5.76	5.77	5.72	5.24	5.07	5.39
6	5.53	5.25	4.79	5.03	4.97	4.57	5.87	5.81	6.03	5.28	5.22	5.39
9	5.33	4.95	5.08	4.79	4.37	4.46	5.87	5.92	5.67	5.17	4.96	5.08
12	5.04	4.58	4.67	4.25	3.92	4.42	5.54	5.46	5.63	4.37	4.50	4.58
15	4.71	4.63	4.71	4.04	4.08	3.92	5.33	5.42	5.33	4.00	4.21	3.58
18	4.10	4.76	4.28	4.26	3.80	3.28	5.40	5.80	5.18	4.59	4.26	4.14
Coefficient of regression	—0.097	—0.080	—0.087	—0.046	—0.092	—0.098	—0.045	—0.015	—0.047	—0.084	—0.080	—0.110
Test of heterogeneity of regression between storage temperatures[1]	F = 0.21			F = 1.59			F = 0.60			F = 0.79		

B. Storage Periods From 0 to 33 Weeks

0	5.40	5.73	5.40	4.80	5.23	5.17	5.57	5.60	5.30	5.05	5.26	5.11
18	5.07	4.10	5.17	4.93	4.00	4.13	5.13	5.07	5.10	5.18	4.80	4.56
21	4.40	4.32	3.47	4.29	4.20	3.70	4.88	5.35	5.08	4.26	4.62	4.48
24	4.15	3.98	3.68	4.24	4.73	3.06	4.23	5.26	4.67	4.62	4.67	4.33
27	4.60	4.40	4.13	4.50	4.14	3.87	4.38	4.56	4.64	4.56	4.47	4.47
30	4.50	3.53	4.07	4.47	3.57	3.87	4.47	5.07	4.93	4.53	4.84	4.23
33	4.35	3.38	3.60	3.96	3.06	3.88	4.75	5.00	5.06	4.53	4.83	4.11
Coefficient of regression	—0.033	—0.066	—0.054	—0.021	—0.054	—0.043	—0.035	—0.021	—0.013	—0.018	—0.016	—0.029
Test of heterogeneity of regression between storage temperatures[1]	F = 1.28			F = 1.32			F = 1.27			F = 0.84		

[1] Five-per cent point for F = 3.68. For method of calculating the test of heterogeneity see Goulden (1939), page 253.

respectively, in contrast to 36—but this does not appear to be responsible, since in the present study the animals did not cause significant variation except at comparatively few storage periods.

Effect of Freezing-Storage on Palatability: The effect of storage upon palatability of the roasts was determined by calculating the regression of the judges' scores for each of the palatability factors upon the time of storage. For these calculations the temperature groupings were disregarded since, as has been shown in the preceding paragraphs, no significant difference had been found among them. The regression coefficients for the various factors are given (Table 3), and the regression lines for some of the factors have been plotted (Fig. 1).

All of the desirability factors—that is, the desirability of the flavor of the exterior fat, interior fat, and lean, and the desirability of the

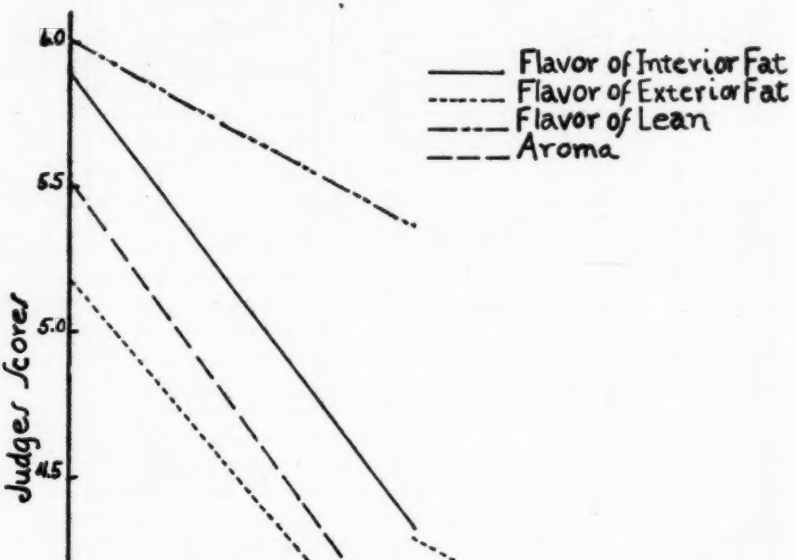

FIG. 1. Regression lines for judges' scores for four desirability characteristics of pork loin roasts on the period of freezing-storage. The two lines for flavor of exterior fat come from different groups of roasts, one group of which was scored triweekly for the entire storage period of 18 weeks and the other from 18 to 33 weeks.

aroma—decreased at highly significant rates for the group of roasts stored 0 to 18 weeks (Table 3). The intensity, however, of none of these factors changed significantly, with the exception of aroma, which increased at a rate significant to the five-per cent level. The tenderness, the richness of juice, and the quantity of juice did not change significantly. For the 0-33 week group, the regression coefficients were calculated on the basis of the 18 through 33 week scores, since this was the period of triweekly scoring. On this basis, the only palatability factors which showed a significant change were the desirability of the flavor of the exterior fat and the richness of juice, both of which decreased at a rate significant to the five-per cent level.

TABLE 3

Coefficients of Regression of Judges' Scores for Palatability Factors on Time of Storage of Pork Roasts

[Storage temperature —18 to —9°C.(0 to 15°F.)][1]

Factor	Stored 0-18 weeks	Stored 0-33 weeks[2]
Desirability of		
Flavor of interior fat	—0.088[3]	—0.044
Flavor of exterior fat	—0.078[3]	—0.035
Flavor of lean	—0.035[3]	—0.017
Aroma	—0.091[3]	—0.015
Intensity of		
Flavor of interior fat	0.001	0.015
Flavor of exterior fat	—0.009	—0.014
Flavor of lean	0.003	—0.009
Aroma	0.031[4]	—0.012
Tenderness	0.012	—0.010
Richness of juice	—0.017	—0.028[4]
Quantity of juice	—0.003	—0.027

[1] One-third of the roasts were stored at —18, another third at —12, and the remaining third at —9°C., but these temperature groupings were disregarded in calculating the regression coefficients given in this table, since previous analyses had shown no significant difference owing to storage temperature. [2] Regression coefficients were calculated on the scores for 18 through 33 weeks, since this was the period of triweekly judging. [3] Significant to the one-per cent level. [4] Significant to the five-per cent level.

Just why more of the factors showed significant rates of deterioration in one group than in the other is not clear. It may have been due to some original dissimilarity between the groups, although the judges detected no significant difference between the fresh samples, except that those representing the 0-18 week group had a more desirable aroma and were less tender than those from the 0-33 week group. The breeds of animals composing the two groups were slightly different (nine Chester White, six Hampshire, two Duroc Jersey, and one Poland China in one group, and seven Duroce Jersey and 11 Poland China in the other), but inspection of the judges' scores for the group containing the four breeds showed no variation attributable to breed; therefore this would not seem to be a reasonable explanation. A more likely cause than either of the preceding would appear to be the difference in method of treatment. (It will be recalled that the second group was stored for 18 weeks without disturbance, after which samples were removed and judged every third week, while for

the first group samples were removed every three weeks from the beginning of storage.) The effect of such prolonged storage without disturbance needs investigation—an attempt, in fact, was made to find its influence upon palatability, but the experiment had to be discontinued after a short time because parts were not available for repairing the refrigerator.

Returning to the data (Table 3) we note, as previously pointed out, that the flavor of the exterior fat decreased in desirability at a significant rate in both groups. The rate for the 0-18 week group was significantly greater than for the other, according to a test for the significance of the difference between the regression coefficients, Goulden (1939). This finding also indicates a difference between the two groups of animals, the possible causes of which have just been discussed.

Maximum Storage Period: It is evident that recommendations regarding a maximum storage period, when based on data such as the foregoing, will necessarily depend upon the degree of desirability accepted as minimum. How low this minimum should be placed is debatable, but for the present discussion the term "slightly desirable," which carries the numerical value of 4.0 on the score sheet used, has been chosen to describe it, since anything less desirable seemed incompatible with the usual advice to freeze only high-grade meat.

Upon this basis, the maximum storage period as judged from the first group of roasts would lie between 15 and 22 weeks, the exact period depending upon whether the exterior fat, aroma, or interior fat is used as the determining characteristic. If the meat is stored without disturbance, there is some evidence from the second group of roasts that this period may be extended to about 26 weeks; these figures may be read from the appropriate curve (Fig. 1). It is interesting to note in this connection that Hiner and Kauffman (1944) report the flavor of the fat in their roasts to have been judged "slightly desirable" at the end of 12 weeks of storage.

Storage, Thawing, and Cooking Losses: The storage and thawing losses from the frozen roasts were very small, the former usually between one and three per cent, the latter less than one per cent of the original weight of the roast (Table 4). The evaporation of moisture during cooking was the greatest single loss, being for the most part around 17 per cent for one group and 14 per cent for the other. The drippings were the next largest, usually seven to eight per cent. The total loss from the frozen roasts during storage, thawing, and cooking was slightly less than the cooking loss from the corresponding fresh roasts.

Press-Fluid and Freezing-Drip: Neither the press-fluid nor freezing-drip changed with storage, but both were slightly higher for frozen roasts than were similar determinations made on the corresponding fresh roasts. The average value of the press-fluid for the frozen roasts was 39.4 per cent of the original weight for the first group, and 38.8 for the second, while the values for the fresh were 36 and 39, respectively. The freezing-drip averaged 12.2 and 12.6 per cent for the two frozen groups, and 10.7 and 11.4 for their corresponding fresh samples.

TABLE 4

Average Losses (in Percentage of Original Weight) Sustained by Pork Roasts During Freezing, Storage, and Cooking

Group No.	Type of loss	Unfrozen	Storage period (weeks)							Average for frozen
			Group 1							
			0	3	6	9	12	15	18	
			Group 2							
			0	18	21	24	27	30	33	
1	Storage	.....	0.8	1.1	1.4	1.3	1.7	1.7	1.9	1.4
	Thawing	.....	0.5	0.6	0.9	0.5	0.6	0.7	0.6	0.6
	Cooking-evaporation	18.9	16.9	16.9	16.8	17.0	16.4	16.1	15.4	16.5
	Drip	10.4	8.8	9.1	8.6	8.5	8.1	7.6	7.8	8.4
	Total	29.3	25.7	26.0	25.4	25.5	24.5	23.7	23.2	24.9
	Total	29.3	27.0	27.7	27.7	27.3	26.8	26.1	25.7	26.9
2	Storage	.....	0.8	2.2	2.7	3.0	3.0	3.2	3.6	2.6
	Thawing	.....	0.5	0.6	0.7	0.7	0.6	0.6	0.7	0.6
	Cooking-evaporation	16.9	14.9	13.5	13.3	13.7	13.0	13.7	13.8	13.7
	Drip	10.5	8.8	6.5	6.1	7.3	7.6	6.4	6.6	7.0
	Total	27.4	23.7	20.0	19.4	21.0	20.6	20.1	20.4	20.7
	Total	27.4	25.0	22.8	22.8	24.7	24.2	23.9	24.7	24.0

SUMMARY

The rate of change in the palatability of pork loin roasts frozen at —18°C. and stored at —18, —12, and —9°C. (0, 10, and 15°F.) has been determined by calculating the regression of judges' scores on storage time. In all, 288 roasts obtained from 36 animals of known feed history and breed were used. These were handled as two groups, one of which was stored for a maximum of 18 weeks, the other for 33. For the first group, the scoring was done triweekly from the beginning of the storage period; for the second, the triweekly scoring was started at the 18th week of storage.

The rates at the different temperatures were not significantly different for any of the factors or for either of the groups, thus indicating that no one of the storage temperatures used preserved the palatability to a greater extent than did either of the others. This finding was further tested by making analyses of variance on the data at each of the scoring periods. The results were confirmatory, for storage temperature was a significant cause of variation at only a few periods, and these were scattered throughout the storage time rather than being concentrated toward the end.

The temperature groupings were then disregarded and the regression coefficients (and lines) were recalculated. All of the coefficients for the desirability factors but none for the intensity factors, except for aroma, were statistically significant for the first group of roasts. (The factors were: flavor of exterior one-eighth inch of fat, flavor of remaining layer of fat, flavor of lean, and the aroma.) For the second group, however, only the coefficient for the desirability of the flavor of the exterior fat was significant. The regression lines for the first group of roasts indicated that flavor of the exterior fat had reached the "slightly desirable" stage after 15 weeks of storage, the aroma after 16, the flavor of the interior fat after 22, and the lean only after an extended period. For the second group of roasts, the time required for the exterior fat to reach the "slightly desirable" stage was 26 weeks.

The frozen roasts lost slightly less moisture and fat during storage, thawing, and cooking than did the corresponding fresh roasts during cooking.

CONCLUSIONS

In so far as could be determined from the triweekly scores of four to five experienced judges on 288 pork loin roasts, which had been frozen at —18°C. and stored 0-18 and 18-33 weeks at —18, —12, and —9°C., no one of the temperatures preserved the palatability to any greater extent than did either of the others.

The flavor of the fat and the aroma decreased in desirability more rapidly with storage than did any of the other palatability factors. The flavor of the lean deteriorated at a moderately slow rate, while the intensity factors, the tenderness, and the juiciness did not change significantly.

According to the standards of the judging panel used, frozen pork loin roasts, even though obtained from high-grade animals and carefully handled, cannot be stored at —18 to —9°C. for longer than 16 to 22 weeks without danger of having the flavor of the fat and the aroma decrease in

desirability beyond the point commonly described as "slightly desirable" and recorded as 4.0 on the score card used.

ACKNOWLEDGMENT

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PHOTOMETRIC ESTIMATION OF VOLATILE SULFUR IN ONIONS AS A CRITERION OF PUNGENCY[1]

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There has been an evident need for a rapid chemical method whereby pungency of onions may be determined. Although knowledge of the essential-oil complex of this plant is not complete, it has been established by Semmler (1892) that sulfur-containing volatile oils are the principal components, and it is permissible to assume that pungency is proportional to volatile sulfur content. This assumption was made by Platenius (1935), who has contributed the only known method designed especially for onions. It involves acid hydrolysis, distillation into saturated bromine water, and determination of volatile sulfur as barium sulfate. Since a period of three days is required to complete an analysis, it is not applicable to routine and control tests, although valuable studies in relation to pungency of onions have been made by its use—Arasimovich and Ivanova (1939) and Platenius and Knott (1934, 1935, 1941). Eaton (1942) employed it in measuring the volatile sulfur content of black mustard plants. It has been the experience of Platenius and of this laboratory that whenever differences in pungency between onion samples were great enough to be detected by taste tests, these differences were shown by the distillation-bromine method.

Methods for determining volatile sulfur in other plants, particularly mustard and rape, have been described. Peterson (1914) heated the tissue at 100°C.(212°F.) in a U-tube, the volatile sulfur being drawn through and absorbed by copper oxide filings. The copper sulfate formed was dissolved in water and the sulfur determined as barium sulfate. Distillation of volatile sulfur compounds into standard ammoniacal silver nitrate solution to form silver sulfide, with back-titration of residual silver with potassium thiocyanate, has been employed in several modifications to determine the volatile sulfur content of plants—Gros and Pichon (1934), and Schuphan (1937). An official A.O.A.C. (1940) method for determining volatile oil in mustard seed is based upon this reaction. These procedures, although more rapid than that involving oxidation with bromine, are inapplicable to onions, since substances other than sulfides which react with the silver distill over.

Information as to the nature of the essential oil of onion derives for the most part from the work of Semmler (1892), who found the principal constituent of the crude oil to be a substance having the formula $C_6H_{12}S_2$, which suggested allyl-propyl-disulfide. He found in addition a higher sulfide with the same radical, and a small amount of a substance seemingly

[1] The writer wishes to thank Mrs. Martha Miller for valuable criticism and cooperation, Mrs. Jessie Jennings for laboratory assistance, Miss Georgiana Flour for analyses of thiamin, and the Basic Vegetable Products Company for permission to publish.

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identical with one of the higher-boiling, sulfur-containing constituents of the asafoetida oils.

Since the sulfur linkage in the onion oil disulfides (R-S-S-R) is similar to that in the amino acid cystine, several colorimetric methods recommended for the latter were investigated. For use in these tests small amounts of crude oil were distilled from Southport White Globe and Australian Brown onions following the method of Platenius (1935) except that the bromine was omitted. Extraction of both distillates with ether gave a brownish-red oil, slightly heavier than water and having a strong odor.

The methylene blue method, involving the so-called Caro's reaction, gave most promising results, and this paper reports on the adaptation of procedures devised for cystine—Toyoda (1934) and Vassel (1941)—to essential oil-sulfur determination in onion. Originally suggested by Emil Fischer, it has been used for hydrogen sulfide in solutions by Freudenberg and Münch (1940), Heath and Lee (1923), Pomeroy (1941), and Suslova (1941); in proteins by Sheppard and Hudson (1930); in sugar by Ambler (1931); in meat and fish by Almy (1925) and Diemair, Strohecker, and Keller (1939); in feces by Lorant and Reimann (1930); for sulfur in iron and steel by Lindsay (1902) and Mecklenburg and Rosenkränzer (1914); and for cystine, but to the writer's knowledge it has not been employed in estimations of sulfur-containing essential oils of plants. The method is based on the reaction between p-amino dimethylaniline hydrochloride and H_2S in the presence of ferric ammonium sulfate to give methylene blue.

Since distillation is avoided, volatile sulfur is not determined as such. This term, however, has been employed by other writers as a criterion of pungency and will be used here as a measure of essential-oil concentration.

EXPERIMENTAL PROCEDURE

The following reagents are used:

p-Amino dimethylaniline monohydrochloride: Dissolve 0.5 gram in 200 ml. of water containing five ml. of concentrated H_2SO_4. Add 45 ml. of additional H_2SO_4 with cooling, and dilute to one liter.

Basic aluminum acetate: Five per cent suspension in water.

Ferric ammonium sulfate: 12.5 per cent in 2.5 per cent by volume H_2SO_4. (113 grams $Fe_2(SO_4)_3(NH_4)_2SO_4.24\ H_2O$ per 500 ml. of solution.)

Sulfuric acid solution: 50 ml. of concentrated acid diluted to one liter.

Sodium sulfide standard: 0.1875 gram $Na_2S.9H_2O$ made to one liter. Use water that has been boiled to remove air. Each milliliter will contain 0.025 mg. of sulfur. Standardize iodometrically.

For Fresh Onions: Place 200 grams of a representative sample (radial sections from at least 20 bulbs) into a Waring blender and add 100 grams of water (recently boiled). Blend for 30 seconds or until a homogeneous pulpy mass is obtained. Pull up into a tared, graduated pipette, the tip of which has been cut off, 12 to 15 grams of the pulp, inserting corks and weighing again. Wash immediately into a one-liter volumetric flask half full of water, add eight ml. of aluminum acetate suspension, and dilute to volume. Water should be as air-free as possible. Allow to stand 30

minutes. Filter 50 to 100 ml. through a No. 1 filter paper into a small Erlenmeyer flask.

To 10 ml. of the filtrate in a small 30-ml. bottle fitted with a plastic or metal cap, add four ml. of the sulfuric acid solution. Sweep out air above the liquid with a stream of nitrogen. Cap tightly and place in autoclave or pressure cooker and maintain at five pounds pressure [109°C. (228.2°F.)] for one hour. Cool and add 7.5 ml. of dimethylaniline solution and 50 mg. of pure zinc powder. Immediately replace cap and agitate slightly to assure even distribution of the zinc. After 30 minutes, remove cap carefully and add two ml. of ferric ammonium sulfate solution by means of a calibrated medicine dropper. Immediately replace cap and invert a dozen times. Set aside for two minutes, then filter through a small plug of fine glass wool inserted in the stem of a small funnel into a 50-ml. volumetric flask. Dilute to volume with distilled water. Measure intensity of color photometrically, using a wave length of 670 mμ, and compute amount of sulfur present by referring to a standard sodium sulfide curve. If a colorimeter is to be employed, a red filter should be introduced into the illuminating system. A reagent blank prepared in the same manner is subtracted from the reading of the sample.

For Dehydrated Onions: Grind onion chips or flakes to at least a 20-mesh powder. Weigh accurately 0.5 gram of the powder into a 250-ml. volumetric flask half full of water. Add two ml. of aluminum acetate suspension, and dilute to volume. Proceed as for fresh onions.

Standard: Prepare a series of dilutions of the sodium sulfide solution so that 10 ml. will contain 0.025, 0.0125, 0.0062, and 0.0031 mg. of sulfur. To the 10-ml. aliquot add four ml. of sulfuric acid solution and 7.5 ml. of dimethylaniline reagent. Add two ml. of ferric alum solution and proceed as directed above.

DISCUSSION OF METHOD

Photometry: A Coleman Universal spectrophotometer, equipped with cylindrical cuvettes with an inside diameter of 16 mm., was employed in the measurements reported in this paper. Spectral-transmittance curves indicate that maximum absorption by the blue-green test solution occurred at 670 mμ (Fig. 1). All readings were made at this wave length, using a Coleman PC-5 filter. In preparing these curves, Na_2S solutions were prepared so that 100 ml. contained 0.302, 0.151, 0.0755, and 0.0377 mg. of sulfur. A fresh onion solution, prepared in the manner described in the procedure, was diluted so that 100 ml. contained 0.175, 0.0875, 0.0700, 0.0525, 0.0350, and 0.0175 mg. of sulfur, based on the standard sodium sulfide curve. Beer's Law is obeyed since straight lines result when optical densities ($\log \frac{1}{\text{transmittance}}$) are plotted against concentration of onion sample, at least up to 32 mg. of onion powder (Fig. 2). The relation held for different weights of sample, dilutions of a single filtrate, and dilution of a single colored solution. The acid concentration must be kept constant for the latter. The reference curve prepared by making dilutions of a standard sodium sulfide solution is shown (Fig. 3).

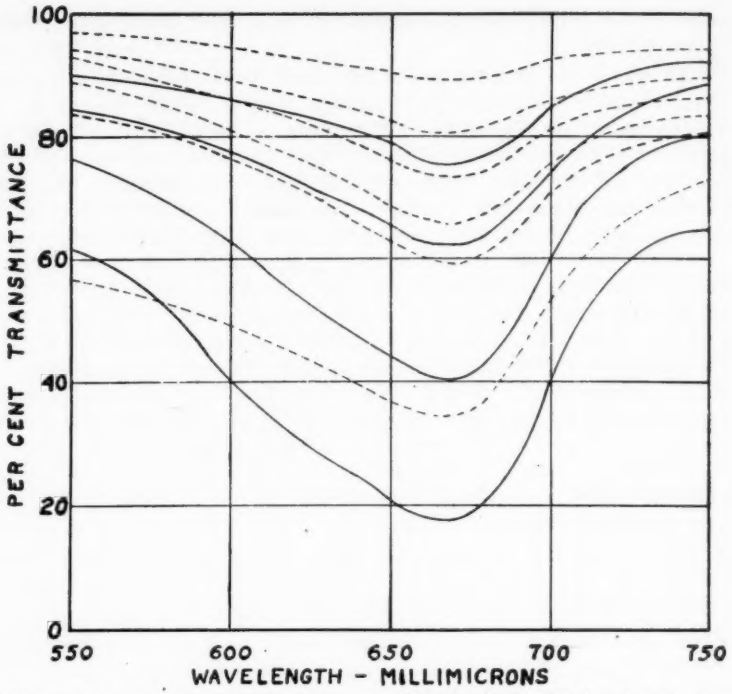

FIG. 1. Spectral-transmittance curves of sodium sulfide (solid lines) and of onion (dotted lines) solutions after development of color.

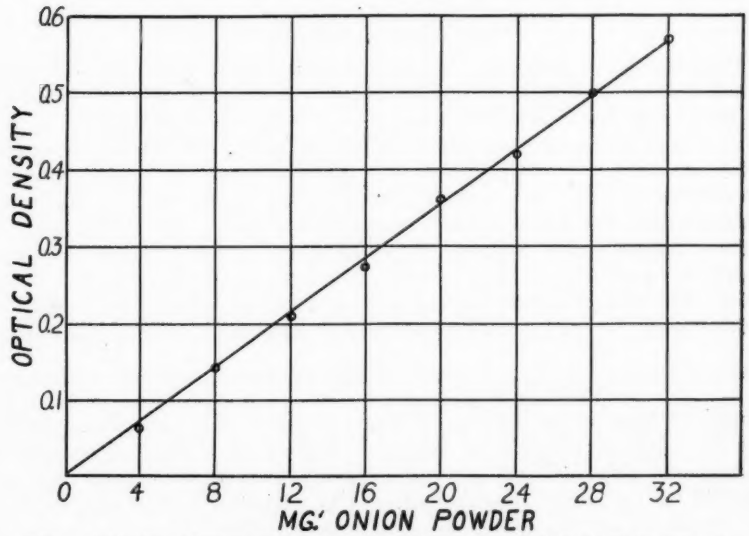

FIG. 2. Relation between optical density and varying weights of onion powder.

Distilled water served as a reference solution in all analyses, so that it was necessary to correct for a reagent blank. The blank reading was fairly constant, amounting to an optical density of approximately 0.025 on the instrument employed.

As a standard, onion oil is not satisfactory, since it is impossible to assume that the distilled oil has a composition identical with that which exists in the tissue. Furthermore there is no assurance that its composition is constant in various types of onions or even among individual bulbs of a single lot. Sodium sulfide solutions have the advantage of ease in preparation and standardization and known purity. They are, however, not stable and must be prepared as needed. Where a colorimeter is used, artificial standards of methylene blue may be desirable, in which case they are standardized against color intensities produced by sodium sulfide solutions.

It is realized that sodium sulfide is an arbitrary standard since it has not been established that this substance displays a reactivity equivalent to that of onion oil, based on volatile sulfur content. Comparisons with the distillation-bromine method, however, suggest an equivalence within reasonable limits. Where comparative values only are sought this relation becomes less important.

Preparation of Sample: The succulent nature of onion-scale tissue permits maceration by a Waring blender to the extent that practically all cells are ruptured in a short time. In this operation the regular blender top was replaced by an adjustable cover composed of a flat piece of wood cut to the shape of the bowl and fitted with an overlapping sheet of rubber. This was pressed down against the onion sections, making an airtight seal, minimizing the volume of air, and considerably decreasing the necessary blending time. Samples were drawn up into a large pipette or piece of large-bore glass tubing through a hole in the cover which was corked during the blending.

A 30-minute extraction period was found sufficient to produce a concentration equilibrium throughout the solution. Tests on 20-mesh onion powder, where blending was omitted, indicated equally rapid extraction.

By using air-free water in making up filtrates, the intercellular air still present in the pulp or powder is dissolved, permitting the fragments to sink to the bottom of the flask.

The amount of sulfur in 10 ml. of sample should not exceed 0.03 mg., since above this limit the method is unreliable. The optimum range appears to lie between 0.01 and 0.02 mg.

Tests using several well-known protein precipitants pointed to basic aluminum acetate as the most suitable. It has been successfully used by Potter and Jones (1943) in separating protein sulfur and sulfide sulfur. It gives a yellow color to extracts of yellow onions, but this does not interfere, the clear filtrate exhibiting at 670 mμ an optical density of zero when compared with distilled water.

Hydrolysis: It has been assumed by Platenius (1935) that the essential oil of onion exists in glycosidal combination, since upon distillation a very low yield of volatile sulfur resulted if the onion mixture was not hydrolyzed with acid. The necessity of hydrolysis was confirmed by the writer.

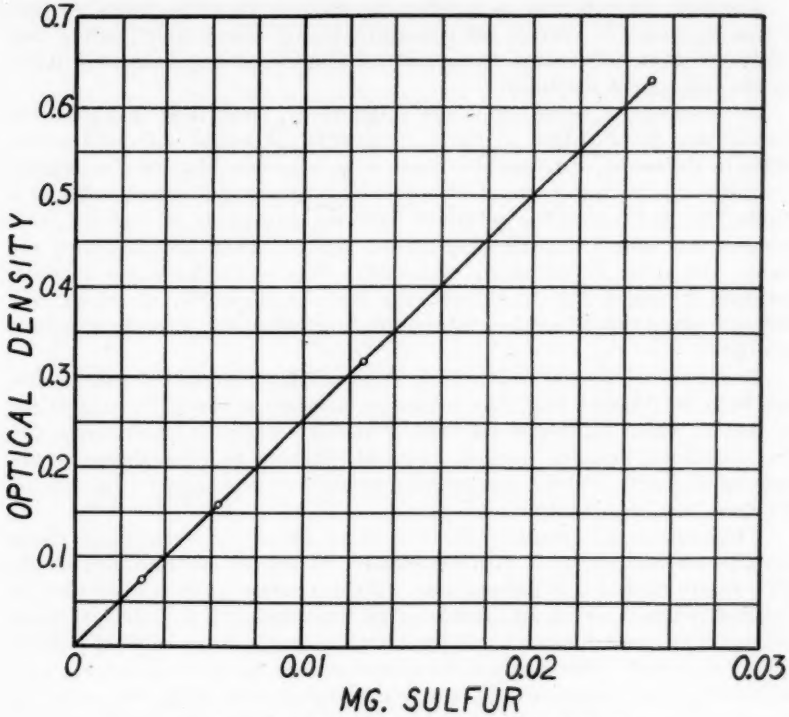

FIG. 3. Standard curve, sodium sulfide solutions.

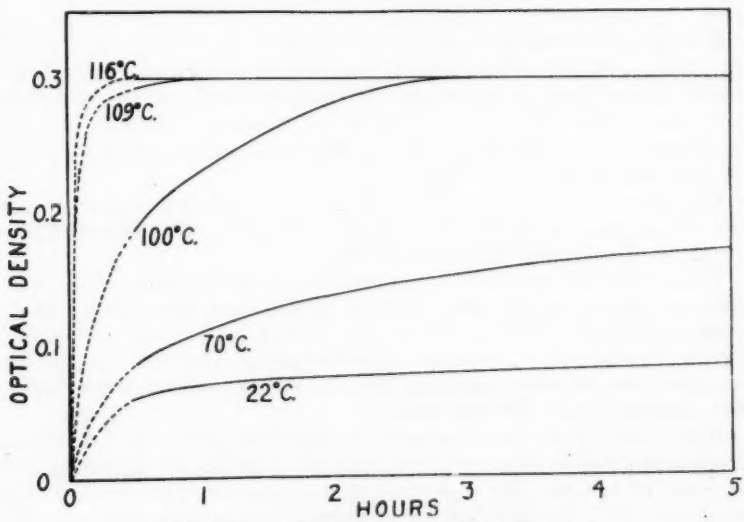

FIG. 4. Rate of hydrolysis with varied temperature.

The amounts of time necessary to insure maximum yield under the conditions specified in the procedure, approximately three hours at 100°C. (212°F.), one hour at 109°C.(228.2°F.), and one-half hour at 116°C. (240.8°F.) are indicated (Fig. 4). Heat alone was ineffective, and acid hydrolysis at temperatures below 100°C. proceeded too slowly.

To prevent leakage from the bottles during heating, circular pieces of high-grade, rubber-sheet packing, previously boiled in alkali to remove any adhering sulfur, were inserted within the bottle cap. The caps were dipped in hot water before closing the bottles to produce a better seal.

After heating, the bottles should be cooled as quickly as possible, avoiding strong light, as prolonged exposure has been found to produce low yields.

Reduction and Color Development: Reduction with zinc dust proceeds rapidly at 22°C.(71.6°F.) and under the described conditions is complete in 15 to 20 minutes. Forty-mesh zinc granules were ineffective, resulting in a yield of about half that obtained from use of zinc powder. C.P. grade powder is recommended, as traces of impurities increase the reagent blank. The 50-mg. portions were measured quickly and rather accurately by means of a small spatula having a hollowed indentation at one end. According to Vassel (1941) zinc here appears to act in two ways—as a reducing agent and as a condensation catalyst.

Development of color is almost immediate. A pink tint appearing at first rapidly disappears. Although filtering is recommended after two minutes, no error is involved if this operation is delayed as much as 10 minutes after introducing the ferric alum. Tests showed that after 15 minutes slight fading occurred. The color of the diluted solution is permanent; it may be measured immediately or the next day.

Losses Owing to Volatilization and Oxidation: Escape of volatile sulfur during blending is minimized by means of the adjustable cover previously described, though a device is desirable which would macerate efficiently without swirling masses of air bubbles into the pulp. No error is incurred in this respect when dried samples are analyzed since blending may be avoided.

There is probably a slight oxidative loss in hydrolyzing the filtrate at the high temperature employed. Both replacement of the air in the bottle above the solution with nitrogen and use of air-free water in preparing the filtrate gave increased yields of one to three parts per million. In older dehydrated onion samples, however, there was a significant decrease in yield when nitrogen was not introduced into the bottles.

Interfering Substances: Cystine and cysteine are known to react in this method. However, qualitative tests [method of Sullivan (1929)] for these substances in the filtrate were negative. Further, the intensity of color developed in the methylene blue method is low compared with sodium sulfide and onion oil. Glutathione reacts negatively by producing a reddish color instead of blue. No protein was detected in the filtrate.

Thiamin reacts rapidly upon reduction in the cold. A fluorometric analysis of thiamin in fresh and dehydrated onions gave values of 0.2 to 0.4 gammas per gram (fresh basis), equivalent to 3.1×10^{-5} to 6.2×10^{-5} mg.

of sulfur in the 10-ml. aliquot employed, which is out of the interfering range.

COMPARISON WITH DISTILLATION METHOD

In order to compare the photometric method with the distillation procedure a series of determinations was carried out on both fresh and dehydrated onions (Table 1). For the fresh material one longitudinally cut quarter of each bulb was divided among duplicate 200-gram colorimetric samples (A and B); opposite quarters plus halves of the fourth quarter constituted duplicate 500-gram distillation samples. Dehydrated samples consisted of well-mixed, 20-mesh powder, 50 grams and 0.5 gram for the distillation and colorimetric methods, respectively. Distillations were performed exactly as described by Platenius (1935) except that for the dehydrated material additional water corresponding to that lost in drying was initially added to the distilling flask.

TABLE 1

Comparison of Results Obtained by Distillation-Bromine and Methylene Blue Methods

Type and variety of onion	Sulfur found[1]					
	Distillation-bromine			Methylene blue		
	A	B	Av.	A	B	Av.
Fresh:						
1. Southport White Globe	131.0	138.0	134.0	132.0	142.0	137.0
2. Southport White Globe	61.3	67.1	64.2	64.6	70.5	67.6
3. Yellow Globe Danvers	153.0	154.0	153.0	163.0	161.0	162.0
4. Yellow Globe Danvers	121.0	.....	121.0	128.0	133.0	130.0
Dehydrated:						
5. Southport White Globe	107.0	105.0	106.0	107.0	109.0	108.0
6. Southport White Globe	101.0	103.0	102.0	110.0	105.0	107.0
7. Southport Yellow Globe	103.0	110.0	106.0	105.0	107.0	106.0
8. White Sweet Spanish	90.5	90.5	90.5	103.0	105.0	104.0
9. Yellow Globe Danvers	96.6	99.2	97.9	.....	.....	94.0[2]

[1] Given in parts per million on fresh-weight basis. [2] Average of eight determinations.

From the results the two methods appear to yield values of approximately the same order. The greater deviation between duplicates in the instance of the fresh samples may be due, at least in part, to sampling differences, since determinations by the colorimetric method on a single filtrate check well. The rather large discrepancy between distillation and colorimetric values in No. 8 of Table 1 is unexplained.

PRECISION AND APPLICABILITY OF THE METHOD

Analysis of a single lot of dehydrated, 20-mesh, Yellow Danvers onion powder yielded data shown (Table 2). Ten weighings of 0.5 gram each were carried through the method simultaneously. The experimental error, based on these results and upon other determinations, appears to lie within $\pm$ three per cent. As mentioned above, the sampling error may be somewhat greater for fresh than for dehydrated material.

Where comparative values of volatile sulfur in fresh and dehydrated onions are desired this procedure is rapid and is suited to routine analysis.

Eight or 10 samples may be handled as a group, requiring an over-all time of four to five hours.

The method is potentially adaptable to analysis of sulfur-containing essential oils in other plants, particularly those of the genus *Allium* and the family *Cruciferae*, since the reaction appears to proceed in a manner similar to that of onion in preliminary experiments.

TABLE 2

Analyses of Yellow Globe Danvers Onion Powder, Indicating Precision of Methylene Blue Method

Sample No.	Optical density readings				Sulfur			Dev. from mean
	A	B	Av.	Av.-blank	In 10-ml. aliquot	Dry basis	Fresh basis	
					mg.	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	.419	.401	.410	.390	.0155	775	91.2	0.0
2	.401	.399	.400	.380	.0151	755	88.8	2.4
3	.415	.417	.416	.396	.0157	785	92.3	1.1
4	.416	.416	.416	.396	.0157	785	92.3	1.1
5	.430	.395	.412	.392	.0155	775	91.2	0.0
6	.419	.....	.419	.396	.0157	785	92.3	1.1
7	.402	.400	.401	.381	.0151	755	88.8	2.4
8	.418	.420	.419	.399	.0158	790	92.9	1.7
9	.411	.399	.405	.385	.0153	765	90.0	1.2
10	.....	.415	.415	.395	.0157	785	92.3	1.1
Blank	.020	.020	.020	.....	.....	.....	91.2[1]	1.5[2]

[1] Mean. [2] Standard deviation.

SUMMARY

A photometric procedure is described whereby pungency of fresh and dehydrated onions may be estimated insofar as this property is dependent on volatile sulfur content. It involves cold extraction with water, acid hydrolysis, reduction with zinc powder, and the reaction with p-amino dimethylaniline hydrochloride to form methylene blue in the presence of ferric ammonium sulfate. The method is rapid, and suitable for routine procedures. It is apparently also adaptable to other plants having sulfur-containing essential oils.

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RELATIVE VALUES OF CAROTENES IN FOODS AS MEASURED BY STORAGE OF VITAMIN A IN LIVERS OF RATS

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The relations between the carotene content of foods and feeds as found by chemical analyses and their biological values as found at various levels of feeding are of considerable scientific and practical importance. Equal quantities of carotene in different foods may not have equal biological values. Smith and Otis (1941) reported that carotenes in certain vegetables have lower values for storage of vitamin A in livers of rats than beta-carotene dissolved in oil. Treichler, Kemmerer, and Fraps (1942) found that carotene in oil is only 50 per cent as effective for storage of vitamin A in livers of rats as equal U.S.P. units in cod liver oil, and that carotene in alfalfa meal was only 21 per cent as effective. The differences may be less when smaller quantities of carotene are fed. At a low level of feeding, Fraps, Treichler, and Kemmerer (1936) reported that carotene in alfalfa products and peanut hay had practically the same vitamin A potency as the carotene in the International standard and in purified commercial carotene dissolved in oil as measured by Sherman-Munsell units. Carotene, as reported in the literature, has been determined by several methods. Kemmerer and Fraps (1943, 1944) have recently reported that such carotene contains impurity A, neo-beta-carotenes B and U, beta-carotene, and sometimes alpha-carotene.

According to Baumann, Riising, and Steenbock (1934) approximately 95 per cent of the total vitamin A of the rat was stored in the liver. The object of the work here presented is to ascertain the differences in biological value between beta-carotene dissolved in oil and the carotenes in various foods as measured by storage of vitamin A in livers of rats, and to find out whether the differences are due to differences in digestibility or to differences in the constituents of the crude carotene or to other causes.

COMPARISON OF CAROTENES IN FOODS WITH CAROTENE DISSOLVED IN OIL

Differences in relative values of carotenes in human foods were compared by the storage of vitamin A in livers of rats. The samples of foods were kept in the freezing compartment of a refrigerator with practically no loss of carotene and vitamin A as shown by weekly analyses. In the analysis of the foods crude carotenes were separated by the A.O.A.C. method (1940), purified by shaking with magnesium carbonate according to the method of Fraps and Kemmerer (1939), and the color read in a KWSZ colorimeter, the product being termed pure carotene. At the time this work was done, the product was supposed to be nearly pure carotene but has since been found to be a mixture of carotenes as stated above.

White rats about 21 days old were divided into five groups of six rats each, equalized as to sex and litter mates and to weight as nearly as

possible, and placed in individual cages. They were fed *ad libitum* a basal ration very low in vitamin A potency, which consisted of white corn meal 37 per cent, wheat gray shorts 25 per cent, cottonseed meal 20 per cent, casein 12 per cent, yeast (irradiated) three per cent, calcium carbonate one per cent, tricalcium phosphate one per cent, and salt one per cent. One group was fed the basal ration without carotene. In the remaining four groups each rat received 60 micrograms of carotenes daily, either from purified commercial carotene dissolved in cottonseed oil or from the foods to be tested. After 14 days the livers were weighed, two or more combined, and the vitamin A determined with a Bausch and Lomb quartz spectrograph by a method similar to that used for butter by Fraps and Kemmerer (1943). The extinction coefficient used was 1600 as in previous work. The term spectro-vitamin A is used to distinguish it from vitamin A determined by other methods, the results by which are expressed in different units.

The spectro-vitamin A so determined includes small amounts of substances which are not vitamin A, according to Baumann and Steenbock (1933), Fraps and Kemmerer (1938), Oser, Melnick, and Poder (1943). Treichler, Kemmerer, and Fraps (1942) found yeast to contain a substance capable of giving a false vitamin A value in livers of rats. Such substances are responsible for part or all of the spectro-vitamin A found in the livers of the rats fed on the basal ration alone.

In each experiment the quantity of spectro-vitamin A in the livers of the rats fed on the basal ration alone was subtracted from the average quantities found in the livers of the other groups, so as to ascertain the net quantities derived from the carotenes in the food or in the purified commercial carotene. The net quantity of vitamin A stored from the commercial carotene in each experiment was used as a standard of comparison for the foods, being given a value of 100. Differences in the spectro-vitamin A stored by the rats on the basal ration and that stored on the standard ration, in addition to differences in the amounts stored from the food being tested, would affect the relative values of the carotenes in the food tested.

The extent of variation in the storage of vitamin A is shown by the data on the rats fed the basal ration and those fed the carotene in oil. In 15 experiments the livers of the six rats in each experiment fed on the basal ration alone averaged 9.5 to 24.8 micrograms of spectro-vitamin A per liver, with an over-all average of 15.5 for the 15 experiments, and a standard deviation of 4.3. In 17 experiments, with commercial carotene in oil, the livers averaged 63.4 to 124.1 micrograms, with an over-all average of 85.9 and a standard deviation of 15.2. These results show that appreciable differences can be expected in the results of experiments made in the same food at different times. The differences are decreased when the averages of several experiments are used, as was done with several foods in the work here reported.

The commercial carotene was purified by solution in chloroform and precipitation with methanol, Fraps and Kemmerer (1939). Chromatographic analyses of the purified commercial carotene showed it to contain 0.8 per cent impurity A, 83 per cent beta-carotene, and 16.2 per cent

alpha-carotene. Since the alpha-carotene has one-half the potency of beta-carotene, 100 micrograms of the standard carotene would be equal to 91 micrograms of beta-carotene. This is termed the beta-carotene equivalent. Chromatographic analyses were not made of the carotenes in the various foods, as the complex nature of the carotene was not known at the time the work was done. Analyses of crude carotene in similar foods were later made by Kemmerer and Fraps (1944), Kemmerer, Fraps, and Meinke (1945), and in subsequent work here reported (Table 4). Neo beta-carotene B has one-half the vitamin A potency of beta-carotene, while impurity A and neo-beta-carotene U do not have vitamin A potency, according to Fraps and Kemmerer (1943). Allowing for the differences between crude carotene and purified carotene, chiefly in impurity A, the beta-carotene equivalents of the foods used were calculated (Table 1).

TABLE 1

Liver Storage of Vitamin A From Carotene of Various Foods as Compared With Carotene in Oil

Food tested	Total spectro-vitamin A in livers	Relative storage of purified carotene		Beta carotene equivalent of carotene determined	Relative storage of beta carotene equivalent
		Individual tests	Average		
	p.p.m.				
Carotene in oil, average	15.5	.....	100	91	100
Apricots, boiled	13.9	49	49	70	64
Apricots, dried, raw	6.0	12	12	70	16
Carrots, cooked	13.9, 11.6	23, 34	29	72	37
Carrots, raw	8.5, 11.6, 6.9	23, 42, 28	31	78	36
Potatoes, sweet, cooked	6.4, 6.4, 6.0, 7.7	10, 14, 17, 13	14	80	16
Pumpkin, canned	6.3, 8.0, 5.1, 6.5	25, 17, 17, 14	18	60	24
Spinach, cooked	10.0	37	37	71	47
Turnip greens	7.6, 6.2, 5.2	20, 12, 14	15	71	19
Alfalfa	14.9	59	59	75	71
Liver, raw beef	32.2, 29.2	196, 130	163	100[1]	179
Butterfat	34.1, 32.2	116, 114	140	100[1]	145
No carotene (average)	4.5	.....	.....	.....	.....
Average of vegetables	.....	.....	.....	.....	32

[1] Vitamin A included.

The total quantities of spectro-vitamin A in the livers and the relative storage in the livers from the different foods are given (Table 1). The data for the individual tests show that appreciable differences may occur between tests made at different times. The average relative storages are compared both on the basis of the purified carotene, equal to 100, and beta-carotene equivalent equal to 100. The latter method gives slightly higher relative values to the carotene of the foods, but both methods agree that the utilization of carotene in foods for storage in livers of rats is much less than that of carotene in oil. Excluding alfalfa, which is not a human food, and butter and liver, which contain vitamin A as well as carotene, the average storage is 32 per cent that of carotene in oil. The relative uncorrected value of 18 for pumpkin, 29 for cooked carrots, 14 for sweet potatoes, 49 for apricots, and 37 for spinach are similar to the relative values of 20, 22, 17, 15, and 58 calculated for the corresponding foods from the data of Smith and Otis (1941).

Butter and liver, which contain vitamin A as well as carotene and are important sources of vitamin A potency, were included in the comparisons. The carotene and the vitamin A in the beef liver fed were determined by the method of Fraps and Meinke (1943) and in the butter by the method of Fraps, Kemmerer, and Meinke (1941) and both were calculated to U.S.P. units. The U.S.P. units were multiplied by 0.6 to obtain the beta-carotene equivalent. The carotene equivalent of butter had an average value of 145, and that of liver, 179, for storage in the rat livers compared with 100 for carotene fed in oil. This is in line with work reported by Treichler, Kemmerer, and Fraps (1942) on cod liver oil, which was found to have twice the value of carotene for storage of vitamin A in rat livers. Booher, Callison, and Hewston (1939) reported that, unit for unit, commercial carotene dissolved in cottonseed oil is only about 50 to 60 per cent as effective as vitamin A in cod liver oil in supporting normal dark adaptation in human adults.

The relative values of the beta-carotene equivalent of beef livers fed were 196 and 130 in the two experiments (Table 1) but there was little difference in the parts per million stored in the livers. The net vitamin A stored per rat liver was 126.7 and 109.5 micrograms for the beef liver and 64.8 and 84.3 micrograms for the standard carotene in oil. These data show that the difference in the relative values found in the two experiments is due, to a greater extent, to differences in the vitamin A stored from the standard carotene given the value of 100 than to differences in the vitamin A stored from the beef liver. Variations in the vitamin A stored from the basal ration and from the standards as well as from the food itself, affect the relative value of the carotene in the food tested.

EFFECT OF OIL ON UTILIZATION OF CAROTENES

Since carotenes are not soluble in water, but are soluble in oil, it was thought that perhaps the addition of oil or fat would aid in dissolving the carotene during digestion and increase the utilization of the carotene. Increased utilization when oil was added was found by Lease (1941) to occur with sweet potatoes fed to rats and by Russell, Taylor, Walker, and Polskin (1942) with carotene in a crystalline form fed to rats.

In the experiments, cottonseed oil was either substituted for part of the corn meal in the basal ration (Table 2) or added directly to the food containing the carotene (Table 3). In each case, 60 micrograms of pure carotene daily per rat in the supplement was fed separately from the basal ration. The addition of oil to the basal ration increased the storage of vitamin A in the livers with the foods used, namely sweet potatoes, spinach, turnip greens, carrots, and pumpkins. With sweet potatoes, the addition of 10 per cent of oil to the ration increased the relative liver storage from 17 to 49. Increase of the oil to 20 per cent gave a further small increase in liver storage from sweet potatoes above the 10-per cent oil level and a more pronounced increase with spinach. Oil added directly to the sweet potato before eating (Table 3) also increased the utilization of the carotene for liver storage. Without oils, in one experiment the relative storage was 10, with 0.1 c.c. of oil it was 17 and with 0.2 c.c. of oil it was 23, com-

pared with 100 for carotene in oil. In the other two experiments the results were similar. The results were also calculated to a beta-carotene basis as shown (Tables 2 and 3). This resulted in slight increase in the relative values of the carotene in the foods.

TABLE 2

Effect of Oil in Basal Rations on Storage of Vitamin A in Rat Livers

Food tested	Oil in basal ration	Total spectro-vitamin A in livers	Relative storage, purified carotene as 100	Relative storage, beta carotene equivalent as 100
	pct.	*p.p.m.*		
Carotene in oil	10	21.0	100	100
Sweet potatoes, baked	0	6.4	17	19
Sweet potatoes, baked	5	9.0	33	38
Sweet potatoes, baked	10	11.7	49	56
Carotene in oil	20	27.4	100	100
Sweet potatoes, baked	10	14.9	48	55
Sweet potatoes, boiled	20	16.8	56	64
Spinach, canned	10	8.7	17	22
Spinach, canned	20	11.4	28	36
Carotene in oil	10	20.2	100	100
Turnip greens	0	6.2	12	15
Turnip greens	5	8.6	19	24
Turnip greens	10	9.0	24	31
Carotene in oil	10	19.6	100	100
Carrots, boiled	0	9.6	34	43
Carrots, boiled	5	11.9	49	62
Carrots, boiled	10	14.4	60	76
Carotene in oil	10	20.5	100	100
Pumpkin, canned	0	6.5	14	21
Pumpkin, canned	5	7.4	19	29
Pumpkin, canned	10	8.9	29	44

TABLE 3

Effect of Oil Added to Sweet Potatoes on Storage of Vitamin A in Rat Livers

Food tested	Total spectro-vitamin A in livers	Relative storage, purified carotene as 100	Relative storage, beta-carotene equivalent as 100
	p.p.m.		
Carotene in 0.1 c.c. oil	17.9	100	100
Sweet potatoes, baked, no oil	5.8	10	11
Sweet potatoes, baked, 1 c.c. oil	6.2	17	19
Sweet potatoes, baked, 0.2 c.c. oil	8.3	23	26
Carotene in 0.1 c.c. oil	18.6	100	100
Sweet potatoes, no oil	6.0	14	16
Sweet potatoes, 0.1 c.c. oil	6.5	16	18
Sweet potatoes, 0.2 c.c. oil	9.1	27	31
Carotene in 0.1 c.c. oil	17.2	100	100
Sweet potatoes, no oil	5.5	13	15
Sweet potatoes, 0.1 c.c. oil	5.9	17	19
Sweet potatoes, 0.2 c.c. oil	7.4	21	24

The results show that fat or oil in the food increases the storage of carotene. A deficiency of fat in the diet resulted in a lower storage of vitamin A in the rat liver. Even in the presence of oil, however, the utilization of carotenes in vegetables for storage of vitamin A in rat livers is much lower than that of carotene in oil. Using the maximum values secured with feeding the oil, the average utilization of carotene in the foods was 44 compared with 100 for carotene in oil and with 22 for corresponding foods in Table 1.

UTILIZATION OF DIGESTED CAROTENES

Experiments were made to ascertain if the low utilization of carotene in foods was due to low digestibility, Kemmerer and Fraps (1938). The procedure used in the experiments was the same as in those just described but, in addition, the feces of the rats were collected daily for 14 days and placed in cold storage. At the end of the period the feces were weighed and crude carotene determined in them and also in the foods. The pigments in the crude carotene were separated by the chromatographic method with the use of calcium hydroxide, Kemmerer and Fraps (1943). The losses in the chromatograph were higher for the feces than is usually the case, and as these losses appeared to be due to destruction of yellow coloring material, not carotene, the results in the feces were not corrected for the losses. The digestibility of the carotenes having vitamin A potency was determined, and the vitamin A stored in the liver was compared on the basis of the digested beta-carotene equivalent.

TABLE 4

Constituents of Crude Carotene

Food tested	Constituents of crude carotene						
	Crude carotene	Impurity A	Neo-beta-carotene U	Beta-carotene	Neo-beta-carotene B	Alpha-carotene	Beta-carotene equivalent
	p.p.m.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Carotene in oil	....	1	....	83	....	16	91
Carrots, raw	98	6	0	53	0	41	73
Carrots, boiled	105	6	0	50	0	44	72
Sweet potatoes, baked	56	12	0	75	13	0	82
Sweet potatoes, baked	45	12	0	68	20	0	79
Turnip greens, canned	39	13	26	51	10	0	56
Spinach, canned	58	11	17	53	19	0	63
Pumpkin, canned	28	12	5	47	8	28	65
Apricots, canned	14	31	0	62	7	0	66
Beet greens, boiled	31	4	12	68	10	....	74

The constituents of the crude carotene in the foods as found by chromatograph separation on calcium hydroxide are given (Table 4). The impurity A and neo-beta-carotene U do not have vitamin A potency, while the alpha-carotene and beta-carotene B have one-half the potency of beta carotene. The beta-carotene equivalent of the pigments having vitamin A potency are given in the table. The word "digested" here means the difference between the quantities of carotene in the food and in the feces.

Part of the carotene may have been destroyed and not assimilated by the rats.

The average percentage digestibilities of the carotenes which have vitamin A potency (Table 5) show that the digestibility of the neo-beta-

TABLE 5

Average Percentage Digestibility of Carotenes[1]

Food tested	Number averaged	Beta	Neo-beta B	Alpha	Neo-beta and beta
Carotene in oil	3	70	....	63	66
Carrots, raw	1	60	....	74	55
Carrots, boiled	1	70	....	49	62
Sweet potatoes, baked	2	53	67	....	56
Turnip greens, canned	1	69	68	....	69
Spinach, canned	1	36	79	....	47
Pumpkin, canned	1	41	19	36	38
Apricots, canned	1	29	0	....	18
Beet greens, boiled	1	56	68	....	58
Average of foods		52	50	53	50

[1] Sixty micrograms per day per rat.

carotene B varied to a greater extent than that of the others. Since there is a possibility that some beta-carotene may be converted to neo-beta-carotene B during the process of digestion, the digestibility of the two combined is also given. The digestibility of the two combined is nearly the same as for beta-carotene, except with apricots and spinach of which the digestibility is much lower. For carrots, turnip greens, and sweet potatoes the digestibility of the beta-carotene and neo-beta-carotene B was nearly the same as that of carotene in oil but for spinach and pumpkins it was much lower. The digestibility of that in spinach appeared quite low, but the storage of vitamin A from spinach in the livers of the rats indicates that this value is too low. The average digestibility of the beta-carotene, neo-beta-carotene B, alpha-carotene, and combined beta and neo-beta-carotene B, was practically the same when all the foods used were considered, being from 50 to 53 per cent compared with 67 per cent for the carotene in oil. Since the digestibilities of the carotenes average about 75 per cent of that of carotene in oil, while the storages in the livers average 32 per cent, it is apparent that the difference in storage is not due to differences in digestibility.

The digestibility of the carotenes in oil is about 67 per cent but the cottonseed oil itself is digested over 90 per cent by rats, Hoagland and Snider (1940). It is a peculiar fact that the carotene is digested to a much smaller extent than the oil in which it is dissolved.

The relative storage of the digested carotene as measured in these experiments is given (Table 6). The rats were fed equal quantities of crude carotene, but since there were differences in the beta-carotene equivalent of crude carotene in the different foods, the data were calculated to the micrograms of spectro vitamin A stored per 100 micrograms of beta-

carotene equivalent digested; from this data, the relative values were calculated as given (Table 6).

Since the carotene in oil contains alpha as well as beta-carotene, and had a beta-carotene equivalent of 91, the results are calculated to relative values compared with beta-carotene. The relative storage compared with beta-carotene ranged from nine to 44 per cent of that of carotene in oil, with an average of 26, although there are some wide differences with individual foods. The average for corresponding foods from the previous experiment (Table 1) is 28.

TABLE 6

Relative Values of Digested Carotene as Measured by Liver Storage of Vitamin A

Food tested	Micrograms of spectro-vitamin A stored in liver per 100 micro-grams of beta carotene equivalent digested	Relative storage from digested beta-carotene equivalent	Relative storage from carotene eaten, from Table 1
Carotene in oil	15.5	100	100
Pumpkin, canned	3.8	26	24
Turnip greens, canned	4.7	33	19
Spinach, canned	6.2	44	37
Carotene in oil	21.6	100	100
Carrots, raw	4.9	26	36
Carrots, boiled	3.7	19	37
Sweet potatoes, baked	1.6	9	16
Average of foods	.....	26	28

The percentage of the digested carotene utilized for storage in the liver was calculated from the data secured. The vitamin A potency of the beta-carotene equivalent of the carotene dissolved in oil may be considered to be 1.67 U.S.P. units per microgram. The spectro-vitamin A of liver may be assumed to be three U.S.P. units per microgram, according to Fraps and Meinke (1943). Using these values and the data secured, it is found that approximately 30 to 40 per cent of the vitamin A potency fed in the carotene dissolved in oil is found in the rat liver, without any allowance for vitamin A used for maintenance. This percentage is higher than the 25 per cent utilized by chickens for storage in eggs, according to Sherwood and Fraps (1935), and the 2.5 per cent utilized by cows for storage in milk, Fraps, Copeland, Treichler, and Kemmerer (1937).

EFFECT OF TOCOPHEROL

Hickman (1943) has pointed out that tocopherol affects the utilization of vitamin A and carotene for gains in weight of rats, the gains being very low if the tocopherol is inadequate. The possibility of a deficiency of tocopherol in the work here presented was tested. The livers of six rats fed 60 micrograms of carotene in spinach with 0.5 microgram of tocopherol per day averaged 46 micrograms of spectro-vitamin A per liver.

compared with 47.3 when tocopherol was not fed; and a similar test with pumpkins gave 37.9 with and 39.1 micrograms without tocopherol. Two additional experiments by Dr. Kemmerer and Mrs. DeMottier on spinach gave similar results. The diets used in this work for spinach and pumpkins evidently contain adequate quantities of tocopherol for utilization of carotene.

DISCUSSION

The difference between the values of carotene fed dissolved in oil and carotene fed in foods may be due to the protective action of the oil against destruction. Hove (1943) found water-soluble agents in the water extracts of the stomachs and livers of rats, which destroy carotene in presence of methyl linoleate. It is possible that oil in larger quantities than was present in the basal ration may have a protective action against such enzymes. When the oil is fed in the ration or with the carotene supplement, a part of the carotene may not be dissolved in the oil, but some of the carotene may be exposed to the enzyme and destroyed. The carotene in the foods, even when oil is fed, is consequently not as effective as carotene dissolved in oil.

SUMMARY

Appreciable differences were found in the quantities of vitamin A stored in livers of young rats from purified commercial carotene fed at different times. In 17 experiments the average total storage from 60 micrograms of carotene per rat per day ranged from 63.4 to 124.1 micrograms per liver, with an average of 85.9 and a standard deviation of 15.2.

In 19 tests on six foods the storage from carotene, on the basis of the beta-carotene equivalent, ranged from 16 to 64 per cent of that of carotene dissolved in oil, with an average of 32.

In four tests with raw beef liver and butterfat the storage from the beta-carotene equivalent of the carotene and the vitamin A averaged 179 and 145 per cent, respectively, of that of carotene in oil.

In 26 tests cottonseed oil increased the utilization of carotene when added to the basal ration or added directly to the carotene supplement. The maximum utilization in four tests was 44 per cent with oil compared with 21 per cent without oil for the corresponding foods, and compared with 100 per cent for the beta-carotene equivalent of the carotene in oil.

The digestibility of the constituents of the carotene in foods having vitamin A activity, including the beta-carotene, beta-carotene B, alpha-carotene, and combined beta-carotene and neo-beta-carotene B, varied with the different experiments but the averages of nine tests were nearly the same, being from 50 to 53 per cent compared with about 70 per cent for carotene dissolved in oil. The digested carotene in foods utilized for storage in the livers of rats, in six tests, ranged from nine to 44 per cent of that of carotene in oil. The average was 26 per cent compared with an average of 28 per cent for carotene eaten in corresponding foods in previous tests when the digestibility was not determined.

The addition of tocopherol to spinach or pumpkins did not increase the storage of vitamin A in the livers.

The lower utilization of carotene in foods as compared with carotene in oil may be due to the protection of the carotene by the oil from the action of destructive enzymes.

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HOME CANNING. I. SURVEY OF BACTERIOLOGICAL AND OTHER FACTORS RESPONSIBLE FOR SPOILAGE OF HOME-CANNED FOODS[1,2]

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An examination of samples of home-canned foods was made to obtain information about the efficiency and safety of home canning, and to learn more about the nature and causes of spoilage. This information is being correlated with heat-penetration, thermal death time, and inoculated-pack data in order to provide additional information on factors concerned in the derivation of procedures for the preparation and processing of home-canned foods which would afford ample protection from botulism poisoning and spoilage, and yield a more appetizing and nutritious product for the home canner.

Since the beginning of the present war, the amount of food canned in the homes of the United States has increased from 2,160,000,000 jars before the war to an estimated 4,600,000,000 to 5,000,000,000 jars in 1943. It is impossible to estimate accurately how much home-canned food was packed in 1944, but it was suggested that the amount would be in excess of 5,000,000,000 jars, Esselen (1944). Consequently, any information which would effect even a modest reduction in spoilage would be of value in the war food program.

Another important aspect of the home-canning program is the ever-existent possibility of botulism poisoning. The extensive victory garden program undoubtedly introduced the art of home canning to many people who, in ordinary times, would never have canned at all. With so many inexperienced people engaged in home canning at a time when pressure canners are not readily available, it is possible that the danger of botulism poisoning from home-canned foods may be increased.

Tanner (1935) and more recently Dack (1943) have reported many of the outbreaks of botulism from home-canned foods which have occurred during the past 40 years. A glance at these records should be convincing evidence that any relaxation of vigilance in processing foods may result in fatal botulism outbreaks. An illustration of the point is the outbreak in New Rochelle, New York, in December, 1943, reported by Hicks (1943), in which three persons were affected. Two of the persons died. The product which caused the illness was an egg plant-pepper mixture, home canned by the open-kettle method.

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[2] Presented at 45th General Meeting of the Society of American Bacteriologists, New York, N. Y., May 3-5, 1944.

TYPES OF SPOILAGE

Most canned-food products are subject to spoilage by molds, yeasts, or bacteria. These organisms may exert their action on foods some time in the interval between harvesting and processing and also occur in containers which (1) have not sealed during processing, (2) have developed leaks after processing, or (3) were underprocessed.

Yeasts and molds in general are low in heat resistance and require oxygen for growth. They are able to grow at relatively low pH values and often cause spoilage in semiacid and acid foods when sufficient oxygen is present in the container.

Foods may be divided into groups according to their susceptibility to each type of spoilage. Cameron and Esty (1940) reported a classification based on the acidity of the product as follows:

(1) Low acid. pH 5.0 and higher. Includes peas, beans, corn, asparagus, marine products, meat, milk, etc. Subject to spoilage by all three groups of thermophiles and by mesophilic putrefactive anaerobes, including *Clostridium botulinum*.

(2) Medium acid. pH 5.0-4.5. Includes meat and vegetable mixtures, spaghetti, soups, sauces whose ingredients produce a partially acid product. Subject to spoilage by the same organisms as group (1). The thermophilic anaerobes (not producing H_2S) which may cause flat sours are of increased importance in this group.

(3) Acid. pH 4.5-3.7. Includes tomatoes, pears, figs, pineapple, nectarines, and other fruits subject to spoilage by nonsporing aciduric types, butyric anaerobes of the *Cl. pasteurianum*, Winogradsky type, and thermophilic anaerobes.

(4) pH 3.7 and below. Includes kraut, pickles, berries, citrus juices, grapefruit, and rhubarb.

REVIEW OF LITERATURE.

The microbiological examination of canned foods is usually undertaken (1) as a direct bacteriological examination of sterility, (2) to investigate the keeping qualities of canned food, based on the presence or absence of viable organisms in the food after processing, and (3) to discover the cause of spoilage (underprocessing, faulty seal, bacterial spoilage, etc.).

Skinner and Glasgow (1919) examined canned asparagus after nine months' storage. Aerobic and anaerobic cultures showed that apparently sound jars often contained *Bacillus subtilis*, *Bacillus vulgatus*, and *Bacillus megatherium*.

Normington (1919) studied cold-packed peas in an effort to find the reason for the high spoilage rate of home-canned foods as compared with commercially canned products. The effects of holding before canning were tested by picking and shelling peas one day and canning the next. Spoilage occurred in 12.1 per cent of the samples which were processed in the autoclave at 15 pounds for 40 minutes on the same day the peas were shelled, and varied from 50-87 per cent (depending upon the method of processing) in samples canned the following day. Normington concluded that the home-canned peas were spoiled by resistant sporeforming organisms not killed by the temperatures maintained in processing. She recom-

mended the use of the pressure canner in conjunction with lengthened process times to insure success.

Lang (1921) examined bacteriologically 938 cans of vegetables, 302 of which were spoiled. About 90 per cent of the samples were processed in the water bath and most were packed in glass containers. Aerobic, anaerobic, and thermophilic bacteria were isolated. Yeasts and molds were observed only in leaky containers. A butyric acid organism isolated from spoiled corn, peas, and lima beans was responsible for 185 of the spoiled samples. Sterility of apparently sound samples varied from 20 per cent for spinach to 80 per cent for tomatoes.

Biester, Weighley, and Knapp (1921) studied the effect of storage temperature on the keeping quality of home-canned vegetables packed by the one-period, cold-pack method. They concluded that process times adequate for one section of the country may not be so for another section and stated that process times may not be considered apart from storage conditions.

Sunderlin, Levine, and Nelson (1928) examined jars of spoiled home-canned foods for appearance, vacuum, odor, pH value, titratable acidity, and amino nitrogen and ammonia. They also made microscopic examinations of the sediment and bacterial counts at 20°C.(68°F.) and 37°C. (98.6°F.) in plates and in dextrose tubes. No one group of tests appeared to be sufficient to detect all cases of spoilage but physical evidences were reported higher than either chemical or bacteriological. Of the tests used, change in odor was the most consistent indication of spoilage.

Richardson and Mayfield (1940) processed peas in the pressure canner at 115.6°C.(240°F.), in the water bath at 95°C.(203°F.), (altitude 5,000 ft.), and in the oven at 121.1°C.(250°F.). There was no spoilage in jars processed from 30 to 50 minutes in the pressure canner but viable organisms were recovered from 9.1 per cent of those processed for 30 minutes. Spoilage ranged from 4.3 to 50 per cent for the jars processed in the water bath for periods ranging from 180 to 324 minutes. Viable organisms were recovered from unspoiled jars processed for 180, 216, 252, and 288 minutes but not from those processed 324 minutes. In oven-canning processes ranging from 90 to 210 minutes spoilage occurred in 16.7 to 90.9 per cent of the jars processed in the oven for periods ranging from 90 to 210 minutes, and viable organisms were recovered from unspoiled jars which were processed 180 and 210 minutes.

EXAMINATION OF HOME-CANNED FOODS

Collection of Samples: The collection of home-canned food samples used in this investigation was accomplished with the assistance of the home demonstration agents of the Massachusetts Extension Service. Samples of sound and spoiled foods were obtained from home canners and brought to the laboratory for examination. About 300 samples were obtained in this way from all parts of Massachusetts.

Information concerning the source, canning, technique, and apparent condition of the sample was obtained by supplying printed labels as shown on the following page to be filled in by the home canner.

1. Canner's name_____ Date_____
2. Address_____
   Product_____
   Product sound (yes or no)_____ Product spoiled (yes or no)_____
   Date spoilage discovered_____
3. Source of raw material:
   Home garden_____ Local produce_____ Shipped in_____
4. Length of time held before canning_____
5. Date packed_____
6. Was this product processed in the water bath (212°F.)?_____
   If so, how long?_____
7. Was it processed in steam pressure?_____
   If so, how long?_____
8. Temperature of storage room (Cool_____ Medium_____ Warm_____)

Treatment of Samples in the Laboratory: Upon arrival at the laboratory the samples were divided into five groups. The obviously spoiled samples and some of the unspoiled samples were examined at once or stored at 4.4° C. (40° F.) until examination was possible. Most of the remaining samples were incubated at 37°C. (98.6°F.) for 30 days prior to examination; some were incubated at 55°C. (131°F.) for 10 days or at both temperatures for 30 and 10 days, respectively. These varied incubation periods were used to indicate the effect of warm storage on the bacteriological findings.

The method used for the examination of samples was essentially that outlined by the Association of Official Agricultural Chemists (1940). This method was formulated for use with commercially canned foods, most of which are packed in tin cans. Practically all the samples received were packed in some type of glass container and most of these were the "lightning-type" glass jar. For this reason, it was necessary to modify the method of opening the containers.

The examination of the container was begun by recording all data which were submitted with the samples. The size and type of container were noted and it was examined for cracks, chips, and evidence of leakage. Foods packed in glass were examined carefully before the containers were opened to determine the presence of gas bubbles, cloudy liquor, sediment, discoloration or disintegration of the product, and any other factors which might indicate the condition of the container or its contents. The rubber rings or sealing compound and glass finish were examined both before and after the container was opened to find any cracks, particles of food, faults in the jar finish, rubber rings, sealing compounds, or sealing surfaces of the cover which might have caused improper seals.

Preparation of Media: Four media were used in the bacteriological examination of both sound and spoiled samples:

1. Liver-liver infusion for anaerobes.
2. Bacto dextrose brom cresol purple agar for flat-sour thermophiles.
3. Bacto nutrient agar for aerobes.
4. Beef heart peptic digest medium for anaerobes.

The liver-liver infusion medium and the beef heart peptic digest were prepared as described in the Association of Official Agricultural Chemists' procedure for the microbiological examination of canned vegetables (1940). The liquid media were exhausted in boiling water for 20 minutes immediately prior to inoculation. Bacto dehydrated media were used in the preparation of the dextrose tryptone brom cresol purple agar and the nutrient agar.

Opening the Containers: Because of the construction of the jars and the presence of rubber rings, no attempt was made to sterilize the tops of the containers by means of a flame. Instead, the container was cleaned with a towel soaked in a five-per cent orthocresol-soap emulsion and subsequently immersed in the cresol emulsion for three to five minutes, after which it was removed and allowed to drain. Then the vacuum was released by inserting a sterile knife blade between the top of the rubber ring and the bottom of the glass cover. Upon being turned slowly the blade lifted the cover slightly, allowing the slow entry of air. Between these operations the knife was immersed in the cresol emulsion.

Sampling: The pipettes used in taking samples for bacteriological analysis were of 10- and 20-ml. capacity. These were used in the inverted position when foods of heavy consistency were sampled. Approximately three grams of sample were pipetted into each of five petri dishes which were poured with dextrose tryptone brom cresol purple agar, and incubated at 55°C.

Two additional petri dishes were inoculated with approximately three grams of sample each. One of the pair was poured with dextrose tryptone brom cresol purple agar and the other with nutrient agar. Both were incubated at 37°C., while cultures made to isolate thermophiles were incubated at 55°C.

Cultures were observed daily for growth, acid production, or gas production and those showing growth were removed from the incubator for microscopic observation and pure culture study.

Thermal Death Time Determinations: In 1920 Bigelow and Esty described the use of small Pyrex tubes inoculated with heat-resistant spores suspended in a medium of known pH value (phosphate buffer or brine of vegetables) for the determination of thermal death times. These tubes were sealed in a flame and heated in a constant temperature oil bath at all the temperatures commonly used in processing foods. Tubes removed from the bath after definite time intervals were cultured to determine the presence or absence of viable spores. Bigelow (1921) found that these time-temperature data, when plotted, indicated an approximately straight-line, semi-log curve. Ball (1923) has used two mathematical characteristics to describe these curves. They are a point and the slope of the curve, further defined as follows:

F = The number of minutes required to destroy the organism (in any specific medium) at 121.1°C.(250°F.).

z = The slope of the thermal death time curve expressed as degrees F. (This is the interval in temperature required for the line to pass through one log cycle of semi-log paper.)

Two other methods are available for the determination of thermal death times. In the first—Townsend, Esty, and Baselt (1938)—the spores are placed in cans two and one-half by three-eighths inches. These cans were designed in the research laboratory of the American Can Company at Maywood, Illinois. The cans are filled with spore suspension (about 13 ml.), sealed under vacuum of about 23 to 26 inches, and heated in small retorts constructed to attain processing temperature quickly.

Williams, Merrill, and Cameron (1937), in the National Canners' Laboratory in Washington, D. C., designed a steam-jacketed tank for the determination of thermal resistance of bacteria. This apparatus has the advantage of determining the rate of destruction of spores at any temperature instead of indicating only survival or destruction points.

Thermal Death Time Procedure: The putrefactive anaerobes isolated were subjected to the temperature of boiling water for 10 minutes to eliminate those of low resistance. The remaining cultures were heated at 110°C.(230°F.) for 30 minutes and those which survived this treatment were used for the thermal death time determinations. The origin of the putrefactive anaerobes used in these tests was as follows:

No. 3679—Canned corn (Reference organism)
No. 19 —Canned lima beans (Amherst, Mass.)
No. 20 —Canned lima beans (Amherst, Mass.)
No. 77 —Canned asparagus (Hinsdale, Mass.)
No. 167 —Canned snap beans (Bradford, Mass.)

The putrefactive anaerobe No. 3679, which was isolated from spoiled corn by Cameron (1927), was included in the group upon which thermal death times were determined. This organism has been studied extensively in connection with safe process times for commercially canned foods. As its heat resistance is well established, it was used here for comparison.

Spore Counts: As the organisms being tested were putrefactive anaerobes, the spore suspensions were prepared by the National Canners' Association method for the preparation of a spore suspension of putrefactive anaerobe No. 3679, Cameron (1943), with some modification.

In making spore counts a special flattened tube, described by Miller, Garrett, and Prickett (1939), was used. Dilutions were made in clarified pork infusion agar. The tubes were incubated at 37°C.(98.6°F.) and the final count was taken when no further increase in the number of colonies was noted.

The spore suspensions, containing 10,000 spores per ml., were used to inoculate phosphate buffer and canned peas, snap beans, and corn brine.

A load of 10,000 spores per No. 2 tin can is used in studying commercial processing times. This load has been reported to give good agreement between processes derived by means of inoculated packs and actual processes and represents a practical maximum load in commercial canning. No comparable quantity has been determined for home-canned foods; consequently the spore suspensions used in this work were standardized at 10,000 spores per milliliter.

The phosphate buffer was prepared by mixing M/15 disodium phosphate with M/15 monopotassium phosphate to give a pH value of 7.0. The

vegetable brines were prepared by decanting the brine from several cans of the vegetables in question, mixing, and resterilizing in an autoclave.

Ten-inch lengths of Pyrex tubing were sealed at one end, filled with two-ml. portions of spore suspension containing 10,000 spores per ml., and sealed in the oxygen flame. These tubes were filled by means of a sterile 20-ml. syringe.

The tubes were then heated in a constant temperature oil bath adjusted to the desired temperature. At specified intervals, five tubes were withdrawn and immersed in ice water until cool.

All tubes were cultured following heating. The tubes were freed from oil, flamed thoroughly in a gas flame, and opened by means of an apparatus similar to the one described by Williams (1929). This consisted of an L-shaped iron bar about $\frac{1}{2}$ x $\frac{1}{16}$ x 8 inches, fastened securely to the laboratory bench. A hole was provided near the end of the assembly through which a thermal death time tube could be inserted. In opening, the tubes were first scratched with a file, flamed thoroughly, and snapped open by inserting through the opening in the iron bar and exerting a slight backward pull. This apparatus was kept flamed at all times to prevent contamination of the tubes.

Cameron (1936) described a modification of Stickel and Meyer's peptic digest medium prepared with liver and beef heart. This medium was used throughout this investigation to determine viability of spores after heating.

Thermal death time determinations were carried out in two parts. In the first, the time intervals at each temperature were widely separated. In this way, approximate points were established at 115.6° C. (240° F.), 110° C. (230° F.), 105° C. (221° F.), and some at 100° C. (212° F.). These runs were then repeated using shorter time intervals at each temperature to determine the final curves.

The survival and destruction points for the five cultures used are shown (Table 9). A one-minute lag was allowed for the temperature in the tubes to reach that of the oil bath. This is in accord with the correction used by Townsend (1939).

RESULTS AND DISCUSSION

Spoiled Samples: The samples reported as spoiled were examined to obtain information regarding the type of spoilage, the bacteriological causes of spoilage, and errors in the use of the canning procedure which may have allowed spoilage to occur.

One hundred and forty-seven spoiled samples were examined, of which 27 samples (18.3 per cent) had been processed in the pressure canner, 88 samples (59.8 per cent) in the water bath, while processing data for the remainder were unknown.

The data obtained include the appearance of the food, pH value, vacuum, odor, bacteriological findings, and bacteriological findings considered with pH value (Table 1). Bacteriological findings indicated the highest percentage of spoilage, followed in order by appearance, vacuum, odor, and pH value. In general there was no consistent relationship between bacteriological findings and pH value considered together.

The types of organisms observed and cultured are shown (Table 2). Microscopic examination showed that 89 (60.6 per cent) of the samples

TABLE 1

Analysis of Data From Spoiled Samples

Product	Number of jars	Process [1]		Number jars in which some factor indicated spoilage						
		P. C.	W. B.	Apparently normal jars	Appearance	Abnormal pH	No vacuum or pressure	Odor	Bacteriological findings	Bacteriological findings and pH
Vegetables										
Asparagus	7	2	3	0	4	3	3	4	7	3
Beans, baked	1	0	1	0	1	0	1	1	1	0
Beans, lima	3	0	3	0	3	0	3	3	2	0
Beans, pea	3	0	1	0	1	0	1	1	1	0
Beans, snap	21	6	13	0	14	11	15	10	20	11
Beets	1	0	1	0	1	0	1	1	1	0
Cabbage	1	0	1	0	1	1	1	1	1	1
Carrots	7	1	4	0	7	3	5	1	5	2
Chard	1	0	1	0	0	1	0	0	1	1
Corn	17	5	11	2	11	5	10	9	15	5
Garden special	4	0	2	1	3	2	3	1	3	2
Greens	7	1	5	0	5	1	6	5	7	1
Peas	5	2	3	0	1	0	2	4	5	0
Pumpkin	1	1	0	0	1	0	1	1	1	0
Rhubarb	1	0	1	0	1	0	1	1	0	0
Spinach	2	0	2	0	1	0	1	1	1	0
Squash, winter	10	0	4	1	8	2	7	1	4	2
Squash, summer	1	0	1	0	0	0	0	1	1	0
Tomato juice	2	0	1	0	2	1	2	1	2	1
Tomatoes	17	3	9	0	13	5	13	10	14	5
Soup mix	1	0	1	0	0	0	0	0	1	0
Fruits										
Applesauce	4	0	1	0	4	0	2	2	4	0
Blueberries	3	0	2	0	1	0	3	2	3	0
Cherries	1	1	0	0	0	0	1	1	1	0
Cherries, white	11	0	1	0	1	0	0	0	0	0
Grape juice	1	0	1	0	1	0	1	1	1	0
Peaches	8	0	6	0	7	0	5	1	7	0
Pears	9	2	5	1	7	0	5	2	8	0
Plums	2	0	2	0	2	1	2	1	1	1
Strawberries	1	0	0	0	1	0	1	0	1	0
Meats										
Beef stew	1	0	1	0	1	0	1	1	1	0
Chicken	3	3	0	0	2	1	3	2	3	1
Special products										
Pickles	1	0	0	0	1	1	1	1	1	1
Special product (composition unknown)	1	0	1	0	1	0	1	1	1	1
Total	147	27	88	5	107	38	102	73	124	38

contained bacteria while 38 (29 per cent) contained molds and 18 (12.2 per cent) contained yeasts. As would be expected, most of the yeasts were observed in the acid products.

Bacterial cultures indicated that 50 samples (34 per cent) contained viable mesophilic aerobes, 49 (33 per cent) contained non-putrefactive anaerobes, 21 (14.2 per cent) contained viable putrefactive anaerobes, and only 13 (6.7 per cent) contained thermophilic or thermoduric organisms.

The apparent causes of spoilage in the samples of low-acid and acid foods, respectively, (Tables 3 and 4) indicate that of a total of 90 samples of low-acid food examined, 63 (81.8 per cent) were spoiled because of understerilization, 14 (18.1 per cent) were spoiled by improper sealing, while 12 (15.5 per cent) were apparently normal. Of the 48 samples of acid foods, 35 (72.9 per cent) were understerilized, 10 (20.8 per cent) had an improper seal, and 3 (6.2 per cent) appeared to be normal. In the underprocessed and spoiled samples, there was no significant difference between those processed in the water bath and those processed in the pressure canner.

Sound Samples: Samples reported as sound were examined to obtain information on the efficiency of the canning procedures used, to find what percentage of the foods reported as sound contained viable microorganisms, and how many of these jars would be likely to spoil during the storage period usually necessary in the home. One hundred and forty-six samples were examined, of which 30 (20.5 per cent) had been processed in the pressure canner, and 92 (63.0 per cent) in the water bath, while for 24 samples (16.4 per cent) the method of processing was unknown.

The examination indicated that 62 samples (42.4 per cent) were sterile. The jars which were not sterile possessed certain characteristics which would indicate spoilage. These were appearance, pH value, lack of vacuum or pressure, odor, and bacteriological findings (Table 5). Bacteriological findings indicated the highest rate of spoilage, followed in decreasing order by pH value, odor, vacuum, and appearance. No general correlation was apparent between the bacteriological findings and the pH value of the food samples.

The types of microorganisms observed with the microscope and the viable forms recovered in culture are shown (Table 6). Four samples (2.7 per cent) contained yeasts, 2 (1.3 per cent) contained molds, and 53 (36.5 per cent) contained bacteria. Bacterial cultures showed that 22 samples (15.0 per cent) contained aerobes, 19 samples (13.0 per cent) non-putrefactive, and 23 samples (15.7 per cent) thermophiles. No putrefactive anaerobes were isolated from the sound samples.

Of 130 jars of low-acid foods (Table 7), 83 samples (63.8 per cent) were apparently sound while 45 samples (34.6 per cent) contained viable organisms which in many instances were potential spoilage organisms or which had already produced some evidence of spoilage.

Of the 45 low-acid samples containing viable organisms 29 (64.4 per cent) were processed in the water bath and 7 (15.5 per cent) in the pressure canner. Of 16 jars of acid foods, (Table 8), 9 samples (56.5 per cent) were apparently sound and 6 samples (36.5 per cent) contained viable organisms.

| | | |

TABLE 2

Bacteriological Findings in Spoiled Food Samples

Product	Number of samples	Number of samples in which organisms were observed			Number of samples from which bacteria were cultured			
		Yeasts	Molds	Bacteria	Mesophilic aerobes	Nonputrefactive anaerobes	Putrefactive anaerobes	Thermophilic aerobes
Vegetables								
Asparagus	7	0	0	7	2	4	0	0
Beans, baked	1	0	0	0	0	1	0	0
Beans, lima	3	0	1	2	0	2	2	0
Beans, pea	1	0	1	1	1	0	1	0
Beans, snap	21	1	3	17	11	8	3	5
Beets	1	0	0	1	0	0	1	0
Cabbage	1	0	0	1	0	0	0	0
Carrots	7	0	1	5	2	4	0	0
Chard	1	0	0	1	1	0	0	1
Corn	17	1	3	14	8	5	4	1
Garden special	4	1	1	2	2	2	0	0
Greens	7	0	1	6	3	3	2	2
Peas	5	0	2	5	2	1	0	0
Pumpkin	1	0	0	1	0	0	0	0
Rhubarb	1	0	0	0	0	0	0	0
Spinach	2	0	1	0	1	1	0	0
Squash	10	1	0	2	2	2	1	0
Squash, summer	1	0	0	1	0	0	0	0
Tomato juice	2	0	1	1	1	0	1	1
Tomatoes	17	1	4	11	2	5	5	2
Veg. mix for soup	1	0	1	1	1	0	0	0
Fruits								
Applesauce	4	4	4	0	0	0	0	0
Blueberries	3	3	1	0	0	0	0	0
Cherries	1	1	0	0	0	0	0	0
Cherries, white	1	0	1	0	0	0	0	0
Grape juice	1	1	1	0	0	0	0	0
Peaches	8	2	2	2	4	5	0	0
Pears	9	1	2	5	2	4	0	1
Plums	2	1	2	0	0	0	0	0
Strawberries	1	0	1	0	1	0	0	0
Meats								
Beef stew	1	0	1	0	0	0	1	0
Chicken	3	0	1	2	2	2	0	0
Special products								
Pickles	1	0	1	0	1	0	0	0
Special product (composition unknown	1	0	1	1	1	0	0	0
Total	147	18	38	89	50	49	21	13

TABLE 3

Causes of Spoilage in Spoiled Low-Acid Foods

Product	Number of jars	Causes of spoilage			Method by which jars showing evidence of spoilage or underprocessing were processed		
		Improper seal	Underprocessing	Apparently normal	Water bath	Pressure cooker	Unknown
Asparagus	7	0	5	2	2	2	1
Baked beans	1	0	1	0	1	0	0
Lima beans	3	1	2	0	2	0	0
Pea beans	1	0	1	0	1	0	0
Snap beans	21	4	16	1	10	6	0
Beets	1	0	1	0	1	0	0
Cabbage	1	0	1	0	1	0	0
Carrots	7	2	5	0	4	1	0
Chard	1	0	1	0	1	0	0
Corn	17	2	9	5	6	3	0
Garden special	4	1	2	1	1	0	1
Greens	7	0	7	0	5	1	1
Pears	5	1	2	2	2	0	0
Pumpkin	1	0	1	0	0	1	0
Spinach	2	1	1	0	1	0	0
Squash	6	0	5	1	4	0	1
Chicken	3	1	2	0	0	2	0
Beef stew	1	0	1	0	1	0	0
Veg. soup mix	1	1	0	0	0	0	0
Total	90	14	63	12	43	16	4

TABLE 4

Causes of Spoilage in Spoiled Acid Foods

Product	Number of jars	Causes of spoilage			Method by which jars showing evidence of underprocessing or spoilage were processed			
		Improper seal	Underprocessing	Apparently normal	Water bath	Pressure cooker	Unknown	Oven
Applesauce	4	0	4	0	1	0	3	0
Cherries	2	0	2	0	0	1	0	0
Grape juice	1	1	0	0	0	0	0	0
Peaches	8	2	5	1	3	0	2	0
Plums	2	2	0	0	0	0	0	0
Blueberries	3	0	3	0	0	0	0	0
Rhubarb	1	0	1	0	1	0	0	0
Tomato juice	1	0	1	0	1	0	0	0
Tomatoes	17	2	14	1	7	3	3	1
Total	48	10	35	3	18	5	11	1

TABLE 5

Analysis of Data From Apparently Sound Samples

Product	Number of jars	Number of jars processed[1]		Number jars apparently normal	Number of jars in which some factor indicates spoilage					
		P. C.	W. B.		Appearance	Low pH	No vacuum or pressure	Odor	Bact. findings	Bact. findings and pH
Vegetables										
Asparagus	17	4	10	12	0	0	0	4	3	3
Beans, baked	1	0	1	0	1	1	1	1	1	1
Beans, lima	3	1	0	0	1	1	0	0	1	0
Beans, snap	56	13	38	28	2	8	5	4	20	6
Beets	2	1	1	0	1	0	0	1	1	0
Broccoli	1	0	1	0	0	0	0	1	0	0
Carrots	7	1	6	2	1	4	1	2	2	2
Corn	19	2	10	6	6	5	3	6	9	4
Garden special	2	0	1	1	0	0	0	0	0	0
Beet greens	1	0	1	1	0	0	0	0	0	0
Greens	7	2	5	1	3	1	2	3	2	0
Mixed vegetables	1	1	0	0	0	0	0	0	1	0
Peas	7	3	3	4	1	0	0	2	2	0
Pig weed	1	0	1	0	0	0	1	0	1	0
Rhubarb	1	....	....	0	0	0	1	0	1	0
Spinach	1	....	....	0	0	0	1	0	1	0
Squash	1	1	0	0	0	0	0	0	1	0
Squash, summer	2	0	2	0	2	0	0	0	1	0
Succotash	2	1	1	0	0	1	0	0	2	1
Tomatoes	5	0	5	4	0	1	1	0	1	1
Tomato juice	2	0	2	1	0	0	1	0	1	0
Tomato soup	1	0	1	1	0	0	0	0	1	0
Wild fern	1	....	....	0	0	0	0	1	1	0
Fruits										
Peaches	2	0	2	1	0	0	0	0	1	0
Pears	1	0	1	0	0	0	0	0	1	0
Raspberries	2	....	....	0	0	0	1	1	1	0
Total	146	30	92	62	18	22	18	27	55	18

[1] P.C.—pressure canner; W.B.—water bath.

In June, 1943, 180 questionnaires were mailed to the home canners who had supplied samples for laboratory examination up to that time. The home canner was asked to estimate how many jars had been canned in 1942, how many remained unused at the time the questionnaire was filled out, and how many had spoiled. Of the 180 cards sent out, 91 were returned. The total number of jars recorded was 22,544 of which 3,739 jars (16.5 per cent) were unused and 443 (1.96 per cent) had spoiled by about June 15, 1943.

TABLE 6

Number of Sound Samples in Which Organisms Were Observed or Cultured

Product	Number of samples	Number samples in which organisms were observed			Number samples from which bacteria were cultured			
		Yeasts	Molds	Bacteria	Mesophilic aerobes	Nonputrefactive anaerobes	Putrefactive anaerobes	Thermophilic aerobes
Vegetables								
Asparagus	17	0	0	10	0	0	0	3
Beans, baked	1	0	0	1	0	1	0	1
Beans, lima	3	0	0	2	1	3	0	0
Beans, snap	56	0	1	20	11	9	0	8
Beets	2	0	0	0	0	0	0	1
Broccoli	1	0	0	0	0	0	0	0
Carrots	7	0	0	4	1	0	0	1
Corn	19	2	0	7	4	3	0	2
Garden special	2	0	0	0	0	0	0	0
Greens, beet	1	0	0	0	0	0	0	0
Greens	7	0	1	2	2	0	0	0
Mixed veg	1	0	0	1	0	0	0	1
Peas	7	0	0	0	0	1	0	1
Pig weed	1	0	0	0	0	0	0	0
Rhubarb	1	0	0	0	0	1	0	0
Spinach	1	0	0	1	0	0	0	0
Squash	1	0	0	0	0	1	0	0
Squash, summer	2	0	0	1	0	0	0	0
Succotash	2	0	0	2	1	0	0	1
Tomatoes	5	0	0	1	0	0	0	0
Tomato juice	2	1	0	0	0	0	0	1
Tomato soup	1	0	0	0	1	0	0	0
Wild fern	1	0	0	0	1	1	0	1
Fruits								
Peaches	2	0	0	0	0	0	0	1
Pears	1	0	0	1	0	1	0	1
Raspberries	2	1	0	0	0	0	0	0
Total	146	4	2	53	22	24	0	23

About 86 per cent of the canners reported spoilage between 0 to 10 per cent, 4.4 per cent reported 10 to 20 per cent spoilage, while only 3.3 per cent reported spoilage in excess of 20 per cent.

Thermal Death Times: The pH values of the phosphate buffer and the vegetable brines in which the spores were heated are shown below:

Medium	pH value
Phosphate buffer	7.0
Pea brine	5.7
Snap-bean brine	5.2
Corn brine	6.1

Total.....
¹ P.C.—pressure canner; W.B.—water bath.

TABLE 7

Condition of Sound Jars of Low-Acid Food

Product	Number of jars	Bacteriological condition			Sound jars			Jars containing viable organisms		
		Apparently sound	Contained viable organisms	Improper seal	Water bath	Pressure canner	Unknown	Water bath	Pressure canner	Unknown
Asparagus	17	13	4	0	9	4	0	4	0	0
Beans, baked	1	0	1	0	0	0	0	1	0	0
Beans, lima	3	2	1	0	0	1	1	0	0	1
Beans, snap	56	35	20	1	26	8	1	12	4	4
Beets	2	1	1	0	0	1	0	1	0	0
Broccoli	1	1	0	0	0	0	1	0	0	0
Carrots	7	3	4	0	2	1	0	4	0	0
Corn	19	12	7	0	5	2	5	5	0	2
Beet greens	1	1	0	0	1	0	0	0	0	0
Greens	7	5	1	1	3	2	0	1	0	0
Mixed vegetables	1	0	1	0	0	0	0	0	1	0
Peas	7	5	2	0	3	2	0	0	1	0
Pig weed	1	1	0	0	1	0	0	0	0	0
Spinach	1	1	0	0	0	0	1	0	0	0
Squash	1	0	1	0	0	0	0	0	1	0
Squash, summer	2	2	0	0	2	0	0	0	0	0
Succotash	2	1	1	0	0	1	0	1	0	0
Wild fern	1	0	1	0	0	0	0	0	0	1
Total	130	83	45	2	52	22	9	29	7	9

TABLE 8

Condition of Sound Jars of Acid Food

Product	Number of jars	Bacteriological condition			Sound jars			Jars containing viable organisms		
		Apparently sound	Contained viable organisms	Improper seal	Water bath	Pressure canner	Unknown	Water bath	Pressure canner	Unknown
Peaches	2	1	1	0	1	0	0	1	0	0
Pears	1	0	1	0	0	0	0	....[1]	0	0
Raspberries	2	1	1	0	0	0	1	0	0	0
Rhubarb	1	0	1	0	0	0	0	0	0	1
Garden special	2	2	0	0	1	0	1	0	0	1
Tomatoes	5	4	0	1	4	0	0	0	0	0
Tomato juice	2	1	1	0	....[1]	0	0	1	0	0
Tomato soup	1	0	1	0	1	0	0	0	0	0
Total	16	9	6	1	7	0	2	2	0	2

Tomatoes	2	1	1	0	[1]	0	0	1	0	0
Tomato juice	2	1	1	0	[1]	0	0	0	0	0
Tomato soup	1	0	1	0	1	0	0	0	0	0
Total	16	9	6	1	7	0	2	2	0	2

TABLE 9

Thermal Resistance of Putrefactive Anaerobes From Canned Foods

Organism No.	Medium	Survival and destruction times at four temperatures							
		240°F.		230°F.		221°F.		212°F.	
		Surv.	Dest.	Surv.	Dest.	Surv.	Dest.	Surv.	Dest.
		min.	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
3679	Phosphate buffer	19	20	43	45	189	191	540	580
3679	Pea brine	20	21	51	53	161	163	560	.....
3679	Bean brine	9	10	16	18	161	163	220	.....
3679	Corn brine	21	22	54	56	197	199	220	.....
167	Phosphate buffer	16	17	54	59	201	203	620	.....
167	Pea brine	17	18	19	21	121	123	380	420
167	Bean brine	5	8	15	17	49	51	.....	.....
167	Corn brine	7	8	35	37	155	157	.....	.....
77	Phosphate buffer	9	10	37	39	163	169	700	740
77	Pea brine	6	7	51	55	141	145	.....	.....
77	Bean brine	0	4	6	8	39	41	80	120
77	Corn brine	7	8	33	35	179	181	.....	.....
19	Phosphate buffer	14	15	33	35	185	189	740	.....
19	Pea brine	9	11	35	37	149	153	700	740
19	Bean brine	4	5	39	41	.....	.....	.....	.....
19	Corn brine	8	9	29	31	159	169	.....	.....
20	Phosphate buffer	13	14	43	45	179	181	680	.....
20	Pea brine	9	11	44	46	149	159	.....	.....
20	Bean brine	3	4	11	15	45	49	.....	.....
20	Corn brine	6	7	40	42	163	169	820	860

The "F" and "z" values of the organisms used in the thermal death time determinations are shown (Table 10); the "F" values range from 0.1 to 6.6 minutes while the "z" values vary between 11 and 23°F.

The length of time necessary for processing at any temperature is dependent upon the thermal resistance of the microorganisms carried by the food. Small changes in thermal resistance may cause a great change in the necessary processing time. The data presented indicate the desirability of further work on the thermal resistance of organisms encountered in home-canned foods. A comprehensive survey is necessary (1) to establish a practical maximum load of spores which may be used in thermal-resistance studies and in inoculated packs, (2) to establish the limits of thermal resistance in spoilage organisms and the average resistance encountered, and (3) to use these data to establish more accurate process times adequate to protect the home canner from spoilage losses and botulism.

TABLE 10

"F" and "z" Values of Heat-Resistant Putrefactive Anaerobes

Organism No.	Phosphate buffer		Pea brine		Snap-bean brine		Corn brine	
	F	z	F	z	F	z	F	z
	min.	*°F.*	*min.*	*°F.*	*min.*	*°F.*	*min.*	*°F.*
3679	5.9	20.0	6.6	21.0	3.4	23.0	5.8	10.0
167	4.5	17.5	6.5	21.0	1.5	19.0	1.4	14.0
77	2.1	15.0	1.8	14.0	0.1	11.5	1.2	13.5
19	3.3	17.0	2.1	16.0	0.7	11.0	1.6	14.5
20	3.4	16.5	2.6	16.5	0.85	17.0	1.4	14.0

SUMMARY AND CONCLUSIONS

Two hundred and ninety-three samples of sound and spoiled home-canned foods were examined in the laboratory by methods adapted from those used in the examination of commercially canned foods.

The samples were examined to determine (1) appearance of container, (2) appearance of food, (3) vacuum or pressure, (4) odor, (5) pH value, (6) bacteria observed with the microscope, and (7) presence of viable microorganisms.

In the laboratory examination of home-canned foods, bacteriological culture tests appeared to be the most reliable criterion of spoilage. More than half of the 146 samples submitted as sound contained viable microorganisms, indicating faulty canning technique and the possibility of spoilage. Ninety-seven per cent of the samples submitted as spoiled gave some evidence of spoilage.

The spores of four putrefactive anaerobes isolated from spoiled jars of asparagus, lima beans, and snap beans survived heating for 30 minutes at 110°C.(250°F.) and 320 to 820 minutes at 100°C.(212°F.). In phosphate buffer their "F" and "z" values were but slightly below those of Cameron's putrefactive anaerobe No. 3679, and indicated a wide range of resistance among the four organisms.

As represented by this investigation, it would appear that about three-fourths of home-canning spoilage is due to understerilization and

one-fourth to improper sealing. The boiling-water-bath method of processing is not adequate to destroy certain types of bacteria encountered in the home canning of low-acid vegetables. While a pressure canner correctly used is satisfactory for processing, understerilization may result if it is misused.

A survey of the spoilage actually encountered by home canners who submitted samples showed that approximately two per cent spoilage was observed by 90 families who canned 22,544 jars of food in 1942. On this basis, the 293 families who submitted samples for laboratory examination canned 73,393 jars of food in 1942 and lost 1,438 jars through spoilage.

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HOME CANNING. II. DETERMINATION OF PROCESS TIMES FOR HOME-CANNED FOODS[1,2]

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This investigation was initiated in order to study home-canning processing times by methods which have been developed, and used successfully, for the determination of process times for commercially canned foods. Considering its importance, but little work has been reported on the application of technical methods for the derivation of home-canning processing times.

In practice home-canned vegetables are processed in a boiling-water bath or a pressure canner, and sometimes in an oven. Bacteriologists are in agreement that the recommended oven and water-bath processing times are inadequate to destroy certain spoilage organisms, including *Clostridium botulinum*. On the other hand, many home canners have objected to the use of pressure canners because the process times, as recommended at present, frequently result in an overcooked product.

The present study, although preliminary in nature, has been carried on in order to obtain a better idea as to the adequacy of boiling-water-bath process times and also to find out whether or not it might be possible to reduce pressure-canner process times and thus provide a safe, but more palatable, product.

REVIEW OF LITERATURE

The determination of a theoretical process time consists essentially of correlating the rate of heat penetration into the canned product during processing and cooling with the lethal rate of the spoilage organism concerned. By this means it is possible to determine a process time and temperature which will provide the desired sterilization value.

Methods of correlating heat penetration and thermal death time data in the derivation of processing times have been described by Bigelow, Bohart, Richardson, and Ball (1920), Ball (1923, 1928), Schultz and Olson (1940), and others.

Studies on heat penetration and processing as related to home-canned foods have been reported by Denton (1918), Skinner and Glasgow (1919), Magoon and Culpepper (1921), Redfield, Nelson, and Sunderlin (1928), Nelson and Berrigan (1939), Nelson and Knowles (1940), Cover, Turk, and Kerns (1943), and others. Nelson and Knowles (1940) and Cover, Turk, and Kerns (1943) recognized the increasing sterilizing effect of air-cooling on glass jars. That the slow air-cooling of glass jars in home

[1] Contribution No. 519, Massachusetts Agricultural Experiment Station, Amherst, Mass.

[2] Presented at 45th General Meeting of the Society of American Bacteriologists, New York, N. Y., May 3-5, 1944.

canning makes a significant contribution to the total sterilizing value of the process is shown in the present investigation.

In the determination of process times for canned foods the theoretical process time, derived from heat penetration and thermal death time data, is generally checked by means of experimental inoculated packs. A general procedure for the use of inoculated packs has been described by Cameron (1931) and by Williams (1940).

EXPERIMENTAL PROCEDURE

Rate of Heat Penetration in Home-Canned Vegetables: The heat-penetration studies were made by means of iron-constantan thermocouples placed in the containers, which were connected through a multiple-pole selector switch to a Leeds and Northrup portable potentiometer.

The thermocouples were prepared by electrically welding the tips of the wire pairs. The first eight inches of the wires were encased in Bakelite tubing of one-quarter inch outside diameter. The wires were separated by inserting a grooved rod of the same material between them in the tube. The thermocouple end of the tube was sealed with Bakelite Cement [3] which was then hardened by drying for two hours at 48.9 to 54.4°C. (120 to 130°F.) followed by baking for four hours at 93.3 to 107.2° C. (200 to 225°F.). The tip of the thermocouple was then coated with can varnish [4] and baked an additional two hours at 93.3 to 107.2°C. The portion of the lead wires which was in the processing equipment was encased in rubber tubing. The ends of the tubing were sealed to the thermocouple and pressure canner lid by means of electricians' rubber tape.

An aluminum pressure canner of 11-quart liquid capacity was fitted with an accurate pressure gauge and mercury thermometer. Four holes were drilled through the cover to admit Bakelite tubes supported by rubber stoppers inserted from the inside. The thermocouple lead wires were led out of these openings to the selector switch. Three pint jars were processed at one time. A small pressure canner and small loads were used so that the process times derived would apply to most of the processing conditions which might be encountered in home canning and apply equally well to processing done in a larger pressure canner containing more jars. The experimental equipment used is shown (Fig. 1).

A large aluminum kettle provided with a wire rack and cover was used as a water bath. Both the pressure canner and the water bath were heated on a one-burner gas plate.

The thermocouples were introduced into the jars by two methods. In the first, "lightning-type" jars were used and the lead wires were forced horizontally through the rubber jar ring. In the second, a screw-top jar with metal lid was used. The lid was fitted with a stuffing box which held the thermocouple in place, as shown (Fig. 2). There was little or no difference in the rate of heat penetration between the two types of jars. The data reported were obtained with jars sealed with metal lids.

[3] Bakelite Cement BC—6031 obtained from Bakelite Corp., Bound Brook, N. J.

[4] Supplied by R. H. Lueck, American Can Company, Maywood, Illinois.

Prior to use the thermocouples were standardized against an accurate mercury thermometer throughout the temperature range used.

In carrying out heat-penetration studies the sealed jars containing thermocouples were placed in the pressure canner, which had two inches of water in the bottom, and the cover was sealed on. The pressure canner was heated rapidly until steam emerged from the open petcock. It was then allowed to vent for seven minutes. This time was sufficient to exhaust the air, as indicated by the thermometer. The petcock was then closed and the pressure was allowed to rise until the desired processing temperature had been reached. The process time was counted from the time the processing temperature was reached.

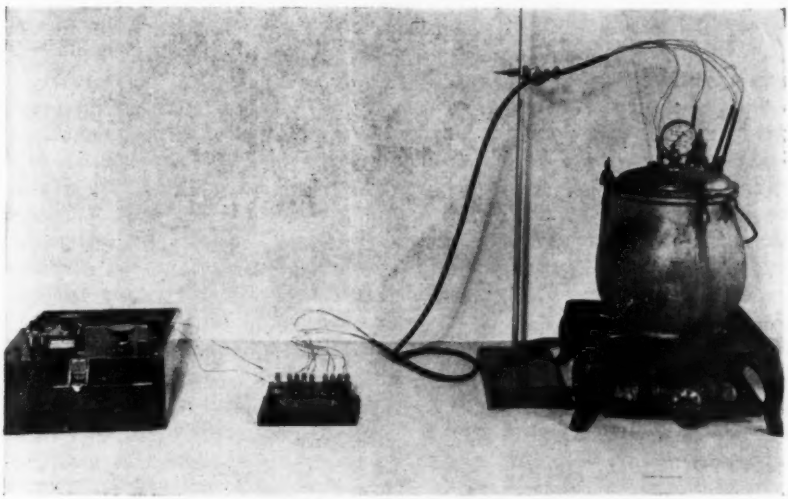

FIG. 1. Apparatus used in heat-penetration studies.

In the boiling-water-bath tests the bath was heated to 82.2°C.(180°F.) and the jars were placed in it. Then the bath was rapidly heated to boiling (in approximately 10 minutes) and the process time was counted from the time the water started to boil. The water boiled at a temperature of 98.9°C. (210°F.) on the days when the tests were made.

The above procedures were set up to approximate adverse home-canning conditions. For this reason initial temperatures were kept low; the initial temperature of the water bath was 82.2°C. instead of boiling, and the pressure canner and water were cold at the start. This was done so the results obtained would be applicable to all canning conditions which might be encountered rather than only the best.

In all tests the temperature readings were taken at three-minute intervals, starting when the jars were first placed in the processing equipment, and continuing throughout the processing period and during cooling until the temperature at the cold point of the jars had reached 82.2°C. At the end of the processing period the jars were removed from the water bath

and placed on a table to cool. Those processed in the pressure canner were removed when the pressure in the canner had dropped to zero. The average cooling period was found to be approximately 12 minutes.

Preparation of Raw Material: The vegetables were prepared according to recommended procedures by Fellers, Chenoweth, and Cole (1943). After blanching they were packed into pint-size home-canning jars. The fill-in

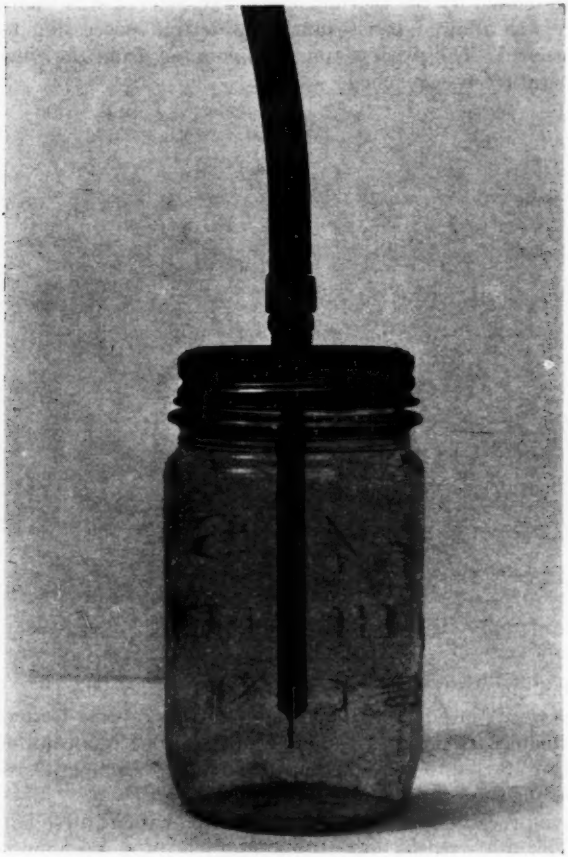

FIG. 2. Method of placing thermocouple in home-canning jars.

weight of all jars of each product was maintained constant. The jars were filled, one-half teaspoon of salt was added to each jar, and they were filled with boiling water, leaving one-half inch head space. They were then sealed with the thermocouple in the desired position. Three jars were processed during each test and the final heat-penetration curve was based on the average curves of six jars.

The Cold Point: In some of the work reported on heat penetration in home-canning containers, the temperature at the center of the container was recorded. It is now well known that the point of slowest heating, or the "cold point," is not usually at the center of the container. The cold point is the point most difficult to sterilize and hence measurements of heat penetration should be made with the thermocouple placed in this position. In products which heat mainly by convection, the cold point is usually near the bottom of the container on the vertical axis, while with conduction heating the point approaches the center of the container.

Thus with all products studied, preliminary heat-penetration tests were made with the thermocouples placed at different locations between the center and bottom of the container, on the vertical axis. The final heat-penetration determinations were made with the thermocouples placed at the cold point.

Heat-Penetration Tests: Average heat-penetration and cooling curves, based on data from six different jars, were obtained for home-canned asparagus, beets, carrots, corn, green beans, peas, spinach, and squash. These data were obtained on jars processed in a boiling-water bath and in a pressure canner at 115.6° C.(240° F.) and 121.1° C.(250° F.). Pint home-canning jars were used in all cases. Based on the data obtained, theoretical process times for the above home-canned vegetables were calculated. The significance of slow air-cooling in home-canning process times was also evaluated.

Inoculated Packs: In order to check the accuracy of the calculated processes range-finding, inoculated packs of corn and green beans were put up and processed both in a boiling-water bath at 98.9°C.(210°F.) and in a home-canning pressure canner at 115.6°C. (240°F.). The methods described by Cameron (1931) and Williams (1940) were followed.

Fresh corn and green beans were prepared for canning in the same manner as for the heat-penetration tests. The blanched vegetables were filled into pint "lightning-type" home-canning jars, to which had been added one-half teaspoon of salt. The filled jars were then inoculated with one milliliter of a spore suspension of Cameron's putrefactive anaerobe No. 3679 which contained 10,000 spores per milliliter. The inoculum was placed in the center of the jars. The jars were then filled with boiling water to within one-half inch of the top, sealed, and processed. A uniform fill-in weight was used in all jars.

All of the inoculated jars in the green-bean packs were subcultured within 24 hours after processing because this vegetable has been found to be a poor medium for the growth of the test organism used. Each jar was thoroughly shaken and a five-milliliter portion of the brine was inoculated into a tube containing 10 milliliters of liver-liver infusion broth. The culture tubes were stratified with two per cent non-nutrient agar and incubated at 37°C.(98.6°F.) for three weeks. Growth of the test organisms was evident from gas production and a putrid odor. The control jars were incubated at room temperature [23.9 to 26.7°C.(75 to 80°F.)] for one week and then for three weeks at 37°C. Bacterial growth was evident from the formation of gas, odor, and cloudiness of the brine.

The corn packs were incubated in the jars at room temperature for one week to eliminate as many spoiled jars as possible. This was followed by incubation for three weeks at 37°C. Growth was evident from odor and gas production.

At the end of the incubation period the control jars of green beans and the jars of corn which had not shown evidence of spoilage, were subcultured in liver-liver infusion broth medium to determine the presence of dormant spores. Both positive jars and cultures were reported as positive in the final results.

In the above inoculated packs only 12 inoculated and six uninoculated control jars were processed in each group. It is realized that a minimum of 24 inoculated and 12 uninoculated containers is recommended for each group. However, available facilities made it necessary to reduce these numbers.

DISCUSSION OF RESULTS

The experimental data (Table 1) were used in the calculation of theoretical process times. In presenting the data, the experimental heating curves obtained are described in terms of their "j" and "f_h" values when plotted on semi-logarithm paper as described by Ball (1928). Based on these data, theoretical process times were calculated by the General Method of Bigelow *et al.* (1920) and by the Modified General Method of Schultz and Olson (1940). By means of the latter method process times at initial temperatures of 37.8°C.(100°F.) and 60°C.(140°F.) were also derived. The results obtained with the two methods were in close agreement and as the latter method permits the determination of process times with different initial temperature, the final results were based on it. The mathematical method of Ball (1928) for calculating process times was not used as it is based upon water cooling after processing and does not apply to the calculation of home-canning processes in which the jars are air-cooled.

Theoretical process times for eight different home-canned vegetables are presented (Table 2). Process times based on initial temperatures of 37.8 and 60°C. are given in order to show the influence of this factor. For comparison, pressure-canner process times at 115.6°C.(240°F.), as recommended by the United States Department of Agriculture and typical boiling-water-bath process times, as recommended by several agencies, are included together with process times as determined by the range-finding, inoculated packs.

The theoretical process times for home-canned vegetables processed at 115.6°C. in a pressure canner are shorter than those recommended at present (Table 2). On the other hand, it is obvious that recommended boiling-water-bath process times would be likely to result in understerilization. These process times are based upon the degree of heating required to destroy the spores (10,000 per pint jar) of Cameron's putrefactive anaerobe No. 3679, with "F" values similar to those used in determining process times for commercially canned foods. At present no evidence is available to indicate whether or not home-canning process times should have the same sterilizing value as used in commercial canning. Much work

TABLE 1

Summary of Heat-Penetration and Processing Data for Home-Canned Vegetables

Product	Fill of container		Initial temp.	Processing temp.	Heating curve		Cooling curve		F value	z value
	Net wt.	Drained			j value	f_h value	j value	f_h value		
	oz.	*oz.*	*°F.*	*°F.*					*min.*	*°F.*
Asparagus	16.5	13.5	60	212	1.00	20.5	1.00	74.5	4.5	18
Green beans	16.5	11.5	93	212	1.00	14.4	1.07	105.5	4.5	18
Sliced beets	17.0	13.0	76	212	.....	.....	1.08	108.0	2.8	18
Sliced carrots	16.5	11.0	77	212	1.06	16.5	1.02	105.0	3.5	18
Corn	16.0	12.0	83	212	1.52	27.5	1.08	68.0	5.6	18
Peas	16.0	11.5	98	212	1.00	21.0	1.08	105.0	5.5	18
Spinach	15.0	13.0	60	212	1.00	45.0	1.04	186.0	4.5	18
Squash	16.0	16.0	74	212	1.46	80.0	1.05	191.0	2.1	18
Asparagus	16.5	13.5	51	240	1.16	14.5	1.06	85.0	4.5	18
Green beans	16.5	11.5	79	240	1.00	18.0	1.20	77.0	4.5	18
Sliced beets	17.0	13.0	74	240	1.00	27.0	1.24	87.0	2.8	18
Whole beets	16.5	12.0	78	240	1.00	22.0	1.17	80.0	2.8	18
Sliced carrots	16.5	11.0	73	240	1.00	18.0	1.18	75.0	3.5	18
Corn	16.0	12.0	93	240	1.06	24.5	1.07	82.5	5.6	18
Peas	16.0	11.5	98	240	1.00	15.0	1.00	83.0	5.6	18
Spinach	15.0	13.0	75	240	1.56	39.0	1.06	78.0	4.5	18
Squash	16.0	16.0	114	240	1.46	54.0	1.18	83.0	2.1	18
Spinach	15.0	13.0	84	250	1.11	52.0	1.32	60.5	4.5	18
Squash	16.0	16.0	92	250	1.44	53.0	1.20	70.0	2.1	18

is necessary before any definite conclusion can be reached. However, in the interest of safe and successful home canning, it appears that it would be well to follow commercial practice in this respect until it has been shown that the sterilizing value of a process can be safely reduced.

The inoculated-pack data for corn and green beans (Table 3) are in general agreement with the theoretical calculated process times as a processing temperature of 115.6°C. However, in the case of the boiling-water bath the green-bean process is shorter and the corn process longer than that determined from heat-penetration data. These differences may be due to the nature of the product, the behavior of the test organism at this processing temperature, and also to the low lethal value of the processing temperature.

TABLE 2

Theoretical Calculated Process Times for Home-Canned Vegetables and Process Times as Recommended at Present

Product	Calculated process times		U.S.D.A. recommended process time	Typical recommended boiling-water-bath process time	Inoculated-pack process time	
	IT[1] 100°F.	IT 140°F.			+	—
PRESSURE-CANNER PROCESS AT 115.6°C.(240°F.)						
	min.	*min.*	*min.*	*min.*	*min.*	*min.*
Asparagus	26.0	24.0	35	.....	.....	.....
Beans, green	25.0	22.5	30	.....	20	25(F=4.5)
Beets, sliced	37.0	34.0	40	.....	.....	.....
Beets, whole	28.5	26.0	40	.....	.....	.....
Carrots, sliced	24.0	22.0	40	.....	.....	.....
Corn, whole kernel	46.0	41.0	65	.....	55	60(F=7.8)
Peas	31.0	30.0	45	.....	.....	.....
Spinach	69.0	66.5	95	.....	.....	.....
Squash	77.0	72.5	85	.....	.....	.....
PRESSURE-CANNER PROCESS AT 121.1°C.(250°F.)						
Spinach	57.0	55.6	60	.....	.....	.....
Squash	61.0	55.5	60	.....	.....	.....
BOILING-WATER-BATH PROCESS AT 100°C.(212°F.)						
Asparagus	.....[2]	606.0	.....	240	.....	.....
Beans, green	.....	616.0	.....	240	255	270(F=2.0)
Beets, sliced	.....	364.0	.....	180	.....	.....
Carrots, sliced	.....	457.0	.....	180	.....	.....
Corn, whole kernel	.....	743.0	.....	180	990	1020(F=7.3)
Peas	.....	745.0	.....	240	.....	.....
Spinach	.....	306.0	.....	240	.....	.....

[1] IT—initial temperature. [2] The slight shift in the calculated water-bath processes owing to the lowered initial temperature is within experimental error.

Theoretical process times for green beans and corn were also derived for four putrefactive anaerobes isolated from home-canned foods as reported previously by Tischer and Esselen (1945). These process times are compared with those obtained experimentally and those based on the lethal rate of the spore suspension of Cameron's putrefactive anaerobe No. 3679 as used in this investigation (Table 4).

TABLE 3

Inoculated-Pack Data

Process time		Process temperature	Inoculated samples showing spoilage [1]	Control samples showing spoilage [1]
		GREEN BEANS		
min.	*hr.*	*°F.*		
.....	1.00	212	12	6
.....	2.00	212	12	2
.....	3.50	212	3	1
.....	3.75	212	5	0
.....	4.00	212	1	0
.....	4.25	212	1	0
.....	4.50	212	0	0
.....	4.75	212	0	0
.....	5.00	212	0	0
.....	5.25	212	0	0
.....	5.50	212	0	0
5	.....	240	9	5
10	.....	240	1	0
15	.....	240	7	0
20	.....	240	1	0
25	.....	240	0	0
		WHOLE KERNEL CORN		
.....	6.0	212	12	6
.....	10.0	212	12	0
.....	10.5	212	12	0
.....	11.0	212	12	0
.....	11.5	212	12	0
.....	12.0	212	11	0
.....	12.5	212	12	0
.....	13.0	212	7	0
.....	13.5	212	9	0
.....	14.0	212	11	0
.....	14.5	212	7	0
.....	15.0	212	3	0
.....	15.5	212	4	0
.....	16.0	212	12	0
.....	16.5	212	1	0
.....	17.0	212	0	0
.....	17.5	212	0	0
.....	18.0	212	0	0
40	.....	240	12	1
45	.....	240	12	1
50	.....	240	12	5
55	.....	240	7	0
60	.....	240	0	0
65	.....	240	0	0

[1] Twelve inoculated and six control jars were processed at each level.

TABLE 4

Calculated Home-Canning Process Times Based on Several Heat-Resistant Food-Spoilage Organisms (Putrefactive Anaerobes)

Product	Organism No.	F value	z value	Theoretical process times [1]	
				IT–140°F. RT–240°F.	IT–140°F. RT–212°F.
		min.	*°F.*	*min.*	*min.*
Beans, green	NCA–3679	3.4	23.0	16	151
Beans, green	MSC–167	1.5	19.0	12	138
Beans, green	MSC–77	0.1	11.5	7.5	196
Beans, green	MSC–20	0.85	17.0	10	144
Beans, green	MSC–19	0.7	11.0	17	2062
Beans, green	.....	4.5 [2]	18.0 [2]	23	584
Corn, whole kernel	NCA–3679	5.8	19.0	40	605
Corn, whole kernel	MSC–167	1.4	14.0	30	730
Corn, whole kernel	MSC–77	1.2	13.5	29	775
Corn, whole kernel	MSC–20	1.4	14.0	30	730
Corn, whole kernel	MSC–19	1.6	14.5	30	754
Corn, whole kernel	.....	5.6 [2]	18.0 [2]	41	746
Peas	NCA–3679	6.6	21.0	28	445
Peas	MSC–167	6.5	21.0	28	439
Peas	MSC–77	1.8	14.0	22	927
Peas	MSC–20	2.6	16.5	22	565
Peas	MSC–19	2.1	16.0	20	524
Peas	.....	5.5 [2]	18.0 [2]	30	714
Phosphate buffer	NCA–3679	5.9	20.0	....	.....
Phosphate buffer	MSC–167	4.5	17.5	....	.....
Phosphate buffer	MSC–77	2.1	15.0	....	.....
Phosphate buffer	MSC–20	3.4	16.5	....	.....
Phosphate buffer	MSC–19	3.3	17.0	....	.....

[1] IT—initial temperature; RT—retort temperature. [2] Theoretical thermal death time data used in calculating processes are shown in Table 1.

TABLE 5

Sterilizing Value of Cooling Curve in Pint Jars of Home-Canned Vegetables Processed in Pressure Canner at 115.6°C.(240°F.)

Product	F value of process	Process time	Actual experimental cooling curve			Twelve-minute cooling curve		
			Time	F value	Sterilizing value [1]	Time	F value	Sterilizing value [1]
	min.	*min.*	*min.*	*min.*	*pct.*	*min.*	*min.*	*pct.*
Asparagus	4.5	24	51.0	1.44	32.0	12	1.40	31.1
Beans, green	4.5	23	15.0	1.67	37.1	12	1.58	35.1
Beets, sliced	2.8	29	12.0	1.00	35.7	12	1.00	35.7
Beets, whole	2.8	26	9.5	0.60	21.4	12	0.62	22.1
Carrots, sliced	3.5	22	12.0	1.20	34.0	12	1.20	34.0
Corn, whole kernel	5.6	41	12.0	1.10	20.0	12	1.10	20.0
Peas	5.5	30	12.0	1.00	18.2	12	1 00	18.2
Spinach	4.5	67	10.0	1.12	24.9	12	1.15	24.6
Squash	2.1	73	6.5	0.36	17.1	12	0.39	18.5

[1] Per cent of sterilizing value of total process.

In general, process times based on the four spoilage organisms isolated from home-canned foods were lower than those based on Cameron's putrefactive anaerobe No. 3679, a standard test organism. These differences vary according to the "F" and "z" values of the organisms in the different vegetables. While it would appear that recommended pressure-canner processing times are more than adequate to destroy these spoilage organisms, it is obvious that in most cases boiling-water-bath process times may be inadequate. The process times derived from these data are interesting because they are indicative of the differences in processing times required to destroy different spoilage organisms which may be encountered in home canning.

As has been pointed out previously, the slow air-cooling encountered in home canning might be expected to contribute significantly to the total sterilizing value of the process. From the data obtained in this investigation the effects of the cooling periods with their original time values and also on the basis of a 12-minute cooling period in a pressure canner have been considered. The sterilizing value of the cooling period from the time the heat was turned off until the temperature at the cold point reached 100°C.(212°F.) has been evaluated in terms of the total process. This information was obtained by means of the General Method for calculating process times and is shown (Table 5). It may be seen that from 18 to 35 per cent of the total sterilizing value of the process for home-canned vegetables, canned in a pressure canner, is contributed by the cooling period after processing is completed. However, in water-bath processing it was found that from 90 to 98 per cent of the lethal value of the process must occur during the actual process as the effect of the come-up and cooling periods is comparatively small.

SUMMARY AND CONCLUSIONS

Theoretical process times for home-canned asparagus, beets, carrots, corn, green beans, peas, spinach, and squash in pint jars were determined for processing temperatures of 100° C. (212° F.) and 115.6° C. (240° F.). Process times based on the heat resistance of Cameron's putrefactive anaerobe No. 3679 were compared with those based on four heat-resistant putrefactive anaerobes isolated from spoiled home-canned foods. The theoretical process times for vegetables processed in a pressure canner at 115.6°C. were found to be somewhat shorter than process times recommended at present. Process times at 100°C. in a boiling-water bath ranged from five and one-half to 12 hours and serve to show that such process times as are recommended at present are inadequate to destroy certain types of bacteria encountered in home canning.

When the pressure canner is used in canning, the relatively long come-up time plus the slow air-cooling period contribute significantly to the sterilizing value of the process. Eighteen to 35 per cent of the sterilizing value occurs during cooling. In the case of the boiling-water bath, 90 to 98 per cent of the lethal value of the process must occur during the actual process since the effect of the come-up and cooling periods is comparatively small.

From experimental evidence it would appear that home-canning process times at 115.6°C., as recommended at present, may be more severe than are necessary and that in many cases they might be reduced. However, before any general recommendations are made, more experimental work must be done and careful consideration given to the many variables encountered. Particular stress should be placed on the correct operation and maintenance of pressure canners in order to obtain the maximum sterilizing value from a given process.

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EXAMINATION OF CANNED APPLE JUICES

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Canned apple juice, both in glass bottles and tin cans, has taken its place on the market with other juices. Like other products of varying acidity it is probably subject to various types of spoilage. It is preserved for the most part by flash-pasteurization for a few seconds (7 to 10) at temperatures ranging from 73.9 to 87.8°C.(165 to 190°F.). Various investigators are of the opinion that since anaerobic conditions prevail after canning or bottling, spoilage by aerobic organisms is very unlikely—Pederson and Tressler (1938), Tressler and Pederson (1938), Arengo-Jones (1940), and others. No mention has been made of anaerobic spoilage.

The problem of tin off-flavor has been recognized and measures have been taken to safeguard the product by using special enamel-lined cans or by bottling. Because of the increased use of glass containers clouding has become a serious problem. It is interesting to study the cause, although most workers are of the opinion that it is centered about changes in the pectin molecule.

The objects of this investigation were to examine chemically, bacteriologically, visually, and by taste and smell various canned apple juices stored two years at room temperatures. Chemical analyses had been made immediately after canning. The problem of clouding was studied in a limited way.

REVIEW OF LITERATURE

According to the literature, heat is the most common method of preserving apple juice; however, according to Carpenter, Pederson, and Walsh (1932), Seitz germ-proof filtering is possible under proper operating conditions. In their study on the preservation of apple juice, Fabian and Marshall (1935) found the pasteurizing times to be determined to a certain extent by the number and types of organisms present as well as by the acidity of the product. Pederson and Tressler (1936) identified spoilage organisms in grape juice as yeasts and vinegar bacteria. Joslyn and Marsh (1937) stated that, although apple juice pasteurized at 60°C. (140°F.) for 30 minutes was rid of organisms and was of better flavor than juice heated at higher temperatures, this treatment did not destroy pectic enzymes. As a result of work previous to 1938, Marshall and Kremer (1937) recommended pouring juice at 87.8°C.(190°F.) into clean, warm, but unsterilized bottles or cans as a safe method of preservation. Beavens, Goresline, and Pederson (1938) found greater killing of microorganisms in pasteurized apple juice cooled gradually after heating than in lots cooled immediately, which they attributed to prolonged heating. Tressler and Pederson (1938) advocated the complete filling of containers since flash-heating did not destroy all mold spores which caused spoilage as long as free oxygen was present; temperatures even as high as 82.2 to

85°C.(180 to 185°F.) for one minute did not destroy mold spores. According to Arengo-Jones (1940) yeasts and bacteria are destroyed by flash-pasteurization at 85°C.; while all mold spores are not killed, they do not develop under anaerobic conditions. Coliform organisms are present in unpasteurized but not in pasteurized citrus juice, according to Nolte and von Loesecke (1940). Esselen, Levine, Fellers, and Strachan (1941) recommended flash-pasteurizing apple juice at 76.7 to 82.2°C.(170 to 180°F.), filling it hot into containers, immediately sealing, and rapidly cooling. According to Tressler and Pederson (1941) the complete filling of containers with juice at the time of pasteurization is necessary in order to prevent spoilage by aerobic organisms, particularly molds.

Concerning spoilage organisms in acid foods, Tanner (1932) reported a ropiness in cider owing to the growth of certain organisms. According to Townsend (1939) a group of butyric acid-producing, sporeforming anaerobes, having a high tolerance for acid and sugar and closely allied in their characteristics to *Clostridium pasteurianum* Winogradsky, are of importance in the spoilage of low-acid canned fruits and tomatoes. Spiegelberg (1940) observed six types of bacterial swells in canned pineapple, three of which were attributed to organisms identified as *Clostridium pasteurianum* Winogradsky, *Leuconostoc mesenterioides* Cienkowski and *Lactobacillus plantarum* (Orla-Jensen) Bergy *et al.* As a part of a study of types of organisms in commercially pasteurized citrus juices in Florida, Nolte and von Loesecke (1940) isolated several yeasts and molds from raw grapefruit juice. Bacteria isolated from pasteurized juice would not grow on orange or grapefruit juice agars under either aerobic or anaerobic conditions at 25, 37, and 55°C. (77, 98.6, and 131°F.). Some of the isolated organisms grew on dextrose agar at 55°C. Pederson and Beattie (1943) stated that, in blending fruit juices with apple juice one cannot always depend upon apple juice to raise the hydrogen-ion concentration sufficiently so that the same pasteurization may be used as for apple juice because of variations in buffer capacity. No correlation between the buffering effect and the ash content of apple juices was observed. Carbonation, according to Fabian and Marshall (1935), has practically no inhibiting effect on bacteria and yeasts.

Clouding of apple juice, according to opinions of various workers, seems to be centered about the disintegration of the pectin molecule. Ehrlich and Schubert (1926) stated that the insoluble residue formed by the action of pectic enzymes under acid condition resembled lignic acid. Kertesz (1930) observed the formation of flocculent precipitates when pectin was hydrolyzed by enzymes in the "pectinase" complex. Carpenter and Walsh (1932) reported the coagulation of colloids in apple juice by rapidly heating to 180°F., holding at that temperature for 20 seconds, and rapidly cooling. Juice thus processed remained clear when bottled. Fabian and Marshall (1935) recommended use of an enzyme (pectinol, a pectin-hydrolyzing enzyme) which when added to apple juice breaks down certain of the colloidal materials responsible for clouding and subsequent precipitation. The predominating colloids present in fruit juices, according to Kertesz (1936), are pectic materials which are converted into an insoluble gel by pectin-methoxylase in the presence of calcium or other

alkaline earth salts. Only three per cent of a pectin solution was precipitated when acted upon by a pectinase enzyme preparation; such a precipitate formed very early in the course of the reaction, and no further precipitate was formed if the pectin solution was then filtered. Marshall and Kremer (1938) observed a roiliness in bottled juice previously heated to approximately 190°F. Juice immediately flash-cooled after flash-pasteurization remained freer from sediment or roiliness than juice bottled hot. According to Pederson and Tressler (1938), varying amounts of sediment were observed in most of several lots of canned apple juice; deaeration had little effect upon the amount. Juices pasteurized at higher temperature than 170°F. contained less sediment. Arengo-Jones (1940) maintains that pectinol breaks down pectin in apple juice, "precipitating part of it and rendering part of it truly soluble." He sometimes obtained perfect stability in apple juices treated with an enzyme, yet in most cases juices so treated precipitated a light sediment after a few months. Pectic enzymes in orange and grapefruit were more active and more heat resistant at pH 4.0 than at 2.2, as reported by Joslyn and Sedky (1940). Mottern, Neubert, and Eddy (1940) found that precipitation in canned apple juice was preceded by the formation of a cloud which usually took place one to six weeks after packing; this cloud disappeared with the formation of the precipitate. They are of the opinion that the cause of precipitation is associated with properties of pectin and that it is not the result of further enzymatic action. Samples of juice to which one per cent of apple pectin had been added just before pasteurization retained body and failed to produce a precipitate. According to these authors, pectinol used for clarifying juices possesses both pectase and pectinase properties; in splitting off methyl alcohol from pectin during clarification, insoluble pectic acid may result. Arengo-Jones (1941) calls attention to the clouding of cider by pectin; the particles of the sediment which forms may vary in size from a fine powder to large, easily discernible flakes. Tressler and Pederson (1941) stated that although flash-pasteurization does not entirely eliminate its deposition, the sediment is scarcely noticeable in the canned or bottled product. Recently Marshall (1943) reported the occurrence of a deposit which developed on the bottoms of containers that had been undisturbed for a few weeks. There was a direct correlation between head space and subsequent precipitation. There was some indication that prompt cooling after pasteurization resulted in less sedimentation than delayed cooling. He recommended the addition of pectin to clarified juice prior to pasteurization to prevent the formation of the insoluble-pectin precipitate, although he did not explain the effect. Neubert (1943) attributed the removal of viscosity-contributing colloidal fractions of juice to sieve action and to absorption by Seitz filter sheets. Centrifugal force was as effective in reducing viscosity and the alcohol-precipitate fraction as filtration through various Seitz filter sheets.

EXPERIMENTAL PROCEDURE

Trees to supply apples were selected in the University orchard at Urbana in 1941 about a month prior to harvesting. Delicious, Golden Delicious, Grimes, Jonathan, Stayman, and Winesap are the most im-

portant fall-maturing varieties of apples grown in this locality and were the varieties selected. The trees ranged in age from approximately 20 to 25 years; they had been moderately pruned, and the fertility of the soil, a brown silt loam, was fairly high. In order to obtain proper ripeness each variety was picked as late as possible. The apples in most cases were put in cold storage on the same day they were picked and maintained at approximately 0 to 1.7°C.(32 to 35°F.). Some time elapsed before all of the apples were pressed.

Each variety was graded into as many classes as possible according to the ordinary defects, and for size and color. Stung apples were apples having more than one superficial codling moth injury per fruit. If an apple had a combination of two injuries, such as scab and worm entrances, it was discarded. Scab was divided into heavy and light infections, depending upon the size and number of lesions. A fruit was classed as being infected with light scab if it had a total area of scab infection less than approximately 20 square millimeters; heavy scab was classed as over five spots per fruit; most of the heavily scabbed fruits were infected with more than five spots per fruit and the total scabbed area was much greater than 20 square millimeters. Apples classed as red were fruits with 75 per cent of the surface colored red; medium red apples had 50 per cent of the surface red; and green fruit had red on less than 50 per cent of the surface. Since it was not possible to find sufficient apples of all varieties with some of the defects, a complete set of samples from all varieties was not obtained.

The apples were washed in a 1¼-per cent solution of hydrochloric acid (by weight) at a temperature of approximately 21°C.(70°F.) to remove spray residue, after which they were rinsed in cold water. They were then ground by means of an apple grater and pressed in the ordinary manner in a hydraulic press. The juices were filtered by a power filter; a special type of diatomaceous earth was used as a filter aid. After filtration, the juices were placed in gallon jugs and kept at approximately 1.1°C.(34°F.) for about 24 hours, and then flash-pasteurized. A stainless-steel or a glass-lined tank was used. After flash-pasteurization for seven to 10 seconds in a block tin coil flash-heater the juice was immediately run into double enamel-lined cans, sealed quickly in an automatic power can sealer, and rapidly cooled by rotating in a special machine under a spray of cold tap water. Each lot of cans was coded for convenience in future handling. The canned samples were stored at ordinary room temperature for three months until analyzed both chemically and microbiologically. Several cans had burst along the seams because of gas formed by yeasts that had not been destroyed by pasteurization.

The following determinations and analyses were made of the juices after they were canned: specific gravity, refractometer reading, viscosity, pH, per cent total acid (expressed as malic acid), and tannin and coloring matter.

Specific gravity was read at 20°C.(68°F.) by means of a hydrometer. The refractometer reading was used to indicate the soluble solid contents. The total milliliters of juice passing through a burette in 45 seconds at a given temperature, divided by the total milliliters of water passing through

the same burette at the same time and temperature, was the value of viscosity. The pH of the juice was determined by means of a Beckman pH meter. For determining total acidities, 10 milliliters of juice were pipetted into a 300-milliliter Erlenmeyer flask, 100 milliliters of water were added, and the contents were titrated against a standard sodium hydroxide of approximately 0.1 normality, using phenolphthalein as the indicator. Water recently redistilled in glass was used, since impurities found in some lots of distilled water gave a difference as high as one milliliter of base. In addition the water was boiled for three minutes to reduce the error owing to the presence of carbon dioxide. With a few modifications the common A.O.A.C. method, given by Jacobs (1938) for the determination of tannin and coloring matter by titration with a standard potassium permanganate solution using indigo carmine solution as an indicator, was used since the determination of the true tannin requires a very involved procedure. Since the juice contained no alcohol, it was not necessary to dealcoholize it by evaporation. Results for the chemical analysis after canning are shown (Table 1).

For microbiological analyses the cans were thoroughly shaken, scrubbed with soap and water, rinsed with distilled water, and dried. The tops were covered with 95 per cent alcohol for about two minutes, the alcohol was burned, and the cans were covered with a sterile Petri plate. The cans were opened by cutting a two-inch hole in the top with a sterile opener designed for that purpose and again covered with the sterile plate. Samples were removed aseptically for counting total dead and live yeast cells with the aid of a Neubauer hemacytometer, after staining the yeast cells for one minute with a 1-to-1,000 concentrated solution of Methylene Blue; for direct microscopic examination of the juice, using the gram-method of staining; and for quantitative plate counts of total numbers of aerobic and anaerobic organisms on dextrose agar, incubated at 37 and 55°C. (98.6 and 131°F.) for 24, 48, and 72 hours. The types of organisms which grew on the medium were in many cases stained and examined microscopically.

The cans were also inspected for swelling, for rust spots, and pitting. The interior was examined for pitting to determine whether there was a correlation between corrosion and swelling. Juices were examined by smell and by taste where possible, and the presence of sediment, clouding, or any abnormal condition was observed and recorded.

For determining anaerobes, Marino's (1907) plates were used. Upon careful examination of plates grown anaerobically, a slight cloudy, spreading growth was observed. This was first visible after 24 hours' incubation at 55°C. but became more definite at 48 hours. It was also observed anaerobically on plates incubated at 37°C. but was not so strong as in plates incubated at 55°C.

Subsurface growth was observed in aerobic plates. In addition, growth occurred at the lower limits of thioglycollate broth in tubes; growth in dextrose broth under seal was very poor and was restricted to the bottoms of the tubes. Gram stains did not distinctly reveal the presence of organisms. Such matter stained gram-negatively, the particles being very small in size and resembling tiny rods.

TABLE 1

Analyses of Juices After Canning

Sample No., variety, and type	Specific gravity	Refr.[1]	Viscosity	pH	Malic acid	Tannin and color[2]
					pct.	
Winesap						
1. L red, stung[3]	1.048	12.0	.912	3.57	.450	.0370
1a. L red, stung	1.048	12.0	.912	3.57	.450	.0370
2. L scab	1.044	11.2	.921	3.65	.410	.0398
3. L russet	1.046	11.6	.924	3.62	.440	.0375
3a. L russet	1.046	11.6	.924	3.62	.440	.0375
4. L rotten	1.040	10.6	.937	3.45	.465	.0565
Golden Delicious						
5. Unsorted	1.049	13.0	.936	3.78	.241	.0302
Stayman (cold storage)						
6. L red (No. 1)[3]	1.045	11.6	.954	3.54	.425	.0480
7. L med. red	1.045	11.6	.944	3.54	.426	.0480
8. L green	1.044	11.2	.942	3.57	.429	.0520
9. M red (No. 1)	1.043	0.2	.924	3.57	.412	.0540
10. M med. red	1.043	11.0	.933	3.62	.364	.0486
11. S green	1.043	11.0	.933	3.59	.425	.0519
Grimes						
12. L yellow (No. 1)	1.044	11.9	.974	3.74	.260	.0541
13. L green	1.042	11.7	.977	3.74	.264	.0581
14. S yellow (No. 1)	1.041	11.0	.971	3.81	.267	.0667
15. S green	1.042	10.9	.974	3.78	.273	.0762
16. L russet	1.046	12.2	.977	3.76	.279	.0603
17. S russet	1.042	11.2	.987	3.78	.293	.0740
18. L scab	1.049	12.6	.987	3.74	.304	.0677
19. L bruise	1.046	12.2	.980	3.78	.257	.0472
20. L wormy	1.046	12.2	.982	3.74	.280	.0677
21. L stung	1.046	12.0	.980	3.74	.280	.0752
22. S scab	1.046	12.0	.982	3.77	.304	.0751
23. S stung	1.043	11.2	.982	3.82	.281	.0802
Delicious						
24. L red (No. 1)	1.046	12.0	.961	4.33	.133	.0385
25. L green	1.041	11.3	.984	4.27	.144	.0455
26. M red (No. 1)	1.041	10.1	.994	4.27	.128	.0458
27. M green	1.040	10.0	.993	4.18	.152	.0457
28. L red, Lt. scab[3]	1.049	12.4	.965	3.98	.160	.0580
29. L red, He. scab[3]	1.055	13.4	.927	4.01	.168	.0472
30. L green, He. scab	1.045	12.4	.985	4.15	.160	.0459
31. M green, Lt. scab	1.041	10.8	.962	4.12	.160	.0454
32. S green, Lt. scab	1.035	9.4	.991	4.15	.152	.0208
33. M red, He. scab	1.051	13.2	.990	4.17	.152	.0615
34. L red, stung	1.046	12.1	.946	4.15	.152	.0338
35. M green, stung	1.039	10.0	.937	4.32	.108	.0435
36. L wormy	1.052	12.8	.937	4.32	.152	.0435
37. Delicious[4]	.....	.....	.....	.....	.....	.....
38. Delicious[4]	.....	.....	.....	.....	.....	.....
39. Delicious[4]	.....	.....	.....	.....	.....	.....
Stayman (room storage)						
40. L red (No. 1)	1.046	11.0	.940	3.70	.377	.0306
41. L med. red	1.042	10.8	.934	3.62	.380	.0289
42. L green	1.040	10.4	.957	3.68	.362	.0247
43. M med. red	1.044	11.4	.948	3.54	.420	.0385
44. M green	1.043	10.9	.942	3.55	.416	.0434
45. L russet	1.045	10.3	.947	3.62	.380	.0422
46. L stung, wormy	1.042	10.8	.950	3.60	.375	.0283
47. Stung	1.050	12.4	.948	3.53	.443	.0434
Jonathan						
48. L red (No. 1)	1.046	11.1	.932	3.62	.376	.0280
49. L green	1.045	11.0	.958	3.56	.368	.0300
50. S red (No. 1)	1.041	10.1	.953	3.61	.320	.0346
51. Under 2½ inches	1.044	11.2	.952	3.57	.360	.0320
52. L red, stung	1.045	11.1	.964	3.52	.393	.0310
53. L red, scab	1.044	11.8	.967	3.54	.393	.0287
54. L red, russet	1.049	12.0	.955	3.49	.440	.0230
55. Del. S red, He. scab	1.050	12.8	.965	4.28	.136	.0638
56. Same as 55	1.050	12.8	.965	4.26	.136	.0638
57. Grimes "drops"[4, 5]	.....	.....	.....	.....	.....	.....
58. Jonathan "drops"[4]	.....	.....	.....	.....	.....	.....
59. Juice, glass packed[4]	.....	.....	.....	.....	.....	.....
60. Juice, glass packed[4]	.....	.....	.....	.....	.....	.....

[1] Refr.—refractometer reading indicating the per cent of sugar. [2] Per cent tannin and coloring matter. [3] L—large; M—medium; S—small. No. 1 indicates No. 1 grade serving as unblemished check. He.—heavy scab; Lt.—light scab. [4] No analyses made on these samples. [5] "Drops"—apples not hand picked but had fallen to the ground.

This condition was studied for a possible relationship between this particular type of growth and clouding. Several lots of juice that had been stored at freezing temperatures for approximately two years were thawed and clarified by filtering through diatomaceous earth. The filtered juice was divided into two main lots, one of which was sterilized at 15 pounds pressure for 30 minutes and the other was not sterilized. Each lot was then subdivided into four lots and treated as follows:

Lot 1 was heated to 190°F., inoculated with the spreading growth from the Marino plates, and heated at 190°F. for two minutes. It was then cooled in about five minutes under the tap. The final temperature was approximately 15.6°C.(60°F.).

Lot 2 was treated in the same manner as Lot 1, but the juice was inoculated with the growth prior to cooling.

Lots 3 and 4 were treated the same as Lots 1 and 2, but were sealed while hot to maintain anaerobic conditions. Noninoculated bottles were used in all four lots as controls.

All lots were then divided and incubated in two series, one at 37°C. and the other at 55°C. Results showed that clouding occurred rapidly only under anaerobic conditions; however, after two weeks a precipitate formed without previous clouding in bottles stored aerobically. In juices incubated anaerobically clouding occurred within 24 hours at 55°C. in the inoculated bottles. The clouding became increasingly pronounced at 48 hours, whereas the noninoculated bottles remained relatively clear. Inoculated juices incubated anaerobically at 37°C. showed clouding at the end of one week. The noninoculated bottles remained clear; after an additional three weeks' storage at room temperatures, however, even the noninoculated bottles began to show slight clouding. If changes of the pectin molecule by hydrolysis were the sole causes of clouding, they should have occurred to the same extent in the control bottles. Furthermore, if deaeration has no effect upon pectin precipitation, as reported by Pederson and Tressler (1938), clouding should have taken place to approximately the same extent under aerobic and anaerobic conditions, which was not the case. It was observed that in juices to which tannin and malic acid equal to twice the original content had been added clouding did not take place. Growth on dextrose agar with a pH of approximately 6, as determined colorometrically, using Bromethymol Blue as the indicator, was better than in dextrose agar having a pH of approximately 7.

Several brands of apple juice were purchased on the market to determine the types of microflora present, and to see if cloudy commercial juices behaved in the same manner as juices canned at this station. These were coded as follows: Lot A, canned in Michigan, was cloudy and packed in brown bottles; Lot B, from Frankfort, Michigan, was cloudy and packed in brown bottles; Lot C, packed in California in green bottles, was "soupy" in appearance; Lot D, Seitz filtered and packed in Frankfort, Michigan, was perfectly clear and came in clear bottles.

These juices were all examined microbiologically and the following results were obtained. Lot A yielded positive anaerobic growth at 37 and 55°C. and no growth under aerobic conditions; Lot B showed no growth under either aerobic or anaerobic conditions at either temperature; Lot D

yielded negative bacteriological results; Lot C gave positive growth under aerobic and anaerobic conditions at both temperatures. Various types of microorganisms were found in this juice. Juice from the last brand was flat in taste.

CONCLUSIONS

The analyses of apple juices after canning are summarized (Table 1); they revealed, in general, the following:

Juices from red apples had a greater specific gravity than juices from green apples; juices from wormy or scabby apples had a greater specific gravity than juice from No. 1 apples; and juices from large apples had a higher specific gravity than juices from small apples.

Apples stored at room temperatures gave juices with higher viscosities than those stored at cold-storage temperatures. Those with more red color yielded juices having higher viscosities than apples with less red color. Juices from apples with stings, worm entrances, and scab lesions had higher viscosities than juices from No. 1 apples. The degree of scab infection influenced viscosity; the heavier the scab, the greater the viscosity.

Juice from fruit with a red color had a higher pH than juice from fruit with a green color. Fruit stored at room temperatures yielded juice having a higher pH than fruit from cold storage. In general, pH did not vary much within the classes of each variety.

Juice from red apples had a higher acidity than juice from green apples. Scabby apples yielded juices with a higher total acidity than No. 1's. Lightly infected scabby apples were lower in acidity than apples more heavily infected. As the size of apples decreased, the acidity of their juices increased. In general, juices from apples with defects had higher acidities than juices from No. 1 apples. There was a relationship between pH and total acidity, but it was not constant.

The tannin-coloring matter content varied with the variety. Juice from green-colored fruit was higher in tannin than juice from red-colored fruit and juices from wormy, stung, and scabby apples were higher in tannin than juices from No. 1 apples. Juice from apples stored at room temperatures had a lower tannin-coloring matter content than that from apples stored at cold-storage temperatures.

It was interesting to compare pH's and total acidities at canning and after storage for two years. In practically all cases hydrogen-ion concentration had decreased but acidity had increased. In addition microorganisms probably had utilized the sugars for energy, releasing organic acids as products, thereby increasing the total titratable acidity. Microorganisms may have attacked the buffer salts, decreasing the hydrogen-ion concentration. The refractometer reading had also decreased. The change in pH and total acidity did not follow the trend present at the time the juices were analyzed after canning. Taking juices from the Delicious variety, for example, No. 28, which was from large, red, light-scab apples, had the lowest pH (3.98) and the second highest acidity (.160) after canning but had the fifth highest pH (4.56) and ranked ninth in total acidity (.203 per cent) at the end of the storage period, for that particular variety.

Regarding the examination of cans externally, results showed that swelling was not always associated with internal pitting. In addition, there apparently was no connection between swelling of cans and the appearance of the juice, or between the internal conditions of cans and the appearance of juice.

Gram stains showed the presence of micrococci, sporeforming and non-sporeforming rods, yeasts, and mold spores. Descriptions of organisms obtained from several samples of juices which had been incubated on dextrose agar showed that all were gram-positive rods. These samples were selected only at random since lack of time prevented examination of all the types of growth present on Petri plates.

Various types of growth were obtained when juices were examined microbiologically at 37°C. Many organisms characterized by spreading growth were found. In addition, *Penicillium* and *Aspergillus* species were found to be viable at the time the investigation was discontinued. One species of yeast was also found.

The most common anaerobic organism was one which manifested itself by a spreading growth, both at 37 and 55°C., although it grew better at 55°C. It was thought that this organism might be connected with clouding; however, the general opinion among workers — Fabian and Marshall (1935), Mottern, Neubert, and Eddy (1940), Arengo-Jones (1941), and Marshall (1943)—maintains that clouding is probably due to changes of the pectin molecule. The first stages of clouding, according to Marshall and Kremer (1937), Pederson and Tressler (1938), and Mottern and Cole (1939), is the appearance of a cloud which gradually precipitates. This clouding had been reported by several workers to take place in about six weeks, as observed in bottles. The belief is that pectase hydrolyzes pectin, splits off methyl alcohol, and forms insoluble pectic acid. Others state that in addition to the known changes which pectin undergoes there exist unknown changes. In addition, Mottern, Neubert, and Eddy (1940) reported that clouding disappears with the formation of a precipitate; if the juice is then filtered no further precipitation occurs. According to results from investigations carried out at this station this was not the case, since clouding occurred several times after filtration through diatomaceous earth. Cloudy juices collected from other sections of the country also clouded after filtration. One brand of juice, processed by the Seitz filtration method without heat, remained perfectly clear after heating and canning. It is probable that enzymes responsible for the breakdown of pectin were not inactivated by this method of filtration; therefore the juice should have clouded, unless the pectin was altered in some undetermined manner. [According to Neubert (1943) the alcoholic precipitate of pectin was decreased when juice was filtered through various Seitz filter sheets.] Pederson and Tressler (1938) reported that deaeration had little effect upon the amount of precipitate formed after canning. Marshall (1943) reported that clouding could be prevented by addition of a small amount of pectin.

It was observed that in some of the cloudy bottles, which were later opened for analysis, mold spores apparently contaminated the contents. They grew luxuriantly and produced a clear zone below the surface of the

mycelial mat in 48 hours. In approximately four days the entire contents became clear. Although it is reasonable to assume that pectic enzymes which molds secrete acted upon the pectin and brought about clearing, the possibility that antibiotic materials produced by the mold may reduce clouding should not be overlooked.

Pectin is probably responsible for clouding, but conditions responsible for this effect have not been fully determined. The facts that growth was observed in juice maintained at 37 and 55° C. anaerobically but not aerobically, and that clouding was very rapid in the inoculated bottles while the noninoculated bottles remained relatively clear should throw more light on this problem.

SUMMARY

Several varieties of canned apple juice stored at ordinary room temperatures for two years were examined chemically and microbiologically. Some cans were normal in appearance; others were springers and still others were flippers.

Changes in pH and total acidity were observed when the experiment was discontinued; however, no close correlation existed. In general, hydrogen-ion concentration decreased while acidity, calculated as per cent malic acid, increased. Refractometer readings decreased.

Pitting was observed in most swelled and normal cans. The extent of pitting varied; it was confined mostly to the seams and bottoms of the cans

Many different types of bacteria and other microorganisms were found which grew on common laboratory media. On the basis of a random selection of colonies growing aerobically on dextrose agar, rod-shaped bacteria were most prevalent.

Clouding was observed in many cases but was not directly accounted for. According to the results obtained, an anaerobic growth may be, at least in part, responsible.

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INCIDENCE AND DISTRIBUTION OF *CLOSTRIDIUM BOTULINUM* IN SOILS OF ILLINOIS

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Botulism was raised from a place of obscurity to a place of prominence owing to outbreaks of the disease resulting from the use of unsafe canning methods during the first World War. The economy of a second World War, with its attendant shortage of critical materials for production of equipment with which to follow safe methods of canning, has re-emphasized the need for studying incidence and distribution of the causative agent *Clostridium botulinum* in soil.

REVIEW OF LITERATURE

The telluric and ubiquitous distribution of *Clostridium botulinum* has been well established by work of a number of investigators. Meyer and Dubovsky (1922a,b,c) Dubovsky and Meyer (1922a,b) Schoenholz and Meyer (1922), Leighton and Buxton (1928), and Scheuber (1929) presented data concerning the occurrence of *Clostridium botulinum* in soils in various parts of the world. Burke (1919a), Coleman (1922), Meyer and Dubovsky (1922a,b) Bachman and Haynes (1924), Hall and Peterson (1924), and Damon and Payabal (1926) made similar investigations of soils in the United States.

The first work of this nature in Illinois was reported by Meyer and Dubovsky (1922b) who examined 14 soil samples and 25 other specimens consisting primarily of plant parts. Twelve and eight-tenths per cent of these specimens produced toxic cultures, two of which were determined to be *Clostridium botulinum* type B. A more extensive investigation of Illinois soils was made by Tanner and Dack (1922), who examined some 56 soil samples, 33 of which were collected from various places around the city of Urbana and 22 from other parts of the state. Of these, 10, or 17.8 per cent, were toxic and on typing were found to contain toxin of *Clostridium botulinum* type B. Graham and Boughton (1924) recovered *Clostridium botulinum* type C from soils of poultry yards in Brown, Christian, Cumberland, and Macon counties where symptoms of botulism had been observed in fowls and also from a barnyard in Green county. *Clostridium botulinum* type C was also isolated by Bengston (1924) from soil samples obtained in Illinois in localities where limberneck in poultry was prevalent.

EXPERIMENTAL PROCEDURE

The procedure followed in preparing samples for culture consisted essentially of transferring aseptically 10 grams of each soil sample to test tubes which were sterilized after the addition of 30 ml. of distilled water and 0.5 gm. of precipitated calcium carbonate to each tube. The test tubes were fitted with sterile one-hole rubber stoppers through which was

passed a short length of glass tubing plugged with cotton, shaken vigorously, and heated in a water bath for 30 minutes at 80°C.(176°F.). Subsequent rapid cooling was accomplished by means of either cold water or air. The sample was again suspended by shaking before transferring it to a flask of liver enrichment medium. After incubation for 10 days at 37°C.(98.6°F.) each culture was tested for toxin production by feeding one ml. of the enrichment culture to a guinea pig.

The glass tube was inserted through the stopper to allow for expansion and contraction of air in the test tube during the heating and cooling processes. To prevent any loss of material during shaking the end of the glass tube was closed in a flame until only a small orifice remained. As most untreated soils in Illinois are distinctly acid, it was desirable to neutralize the soil samples prior to heating. Addition of calcium carbonate to the tubes proved to be the most convenient and satisfactory method of obtaining a pH value in the range of neutrality.

The first 100 soil samples received were examined in a more thorough manner than those just described. Enrichment cultures of each sample were prepared in duplicate. The soil inoculum of one culture was heated as mentioned above but the inoculum of the other culture was not heated. After incubation for 10 days at 37°C. these enrichment cultures were tested for toxicity by feeding one ml. of each culture to guinea pigs. Glucose agar shake cultures were prepared from enrichment cultures, and five separate colonies, presumably typical of *Clostridium botulinum*, were picked from each shake culture and placed in thioglycollate medium. After incubation at 37°C. for the same period of time these latter cultures were also tested for toxin-production by feeding one ml. of each culture to guinea pigs.

A liver infusion medium with liver particles was selected as the enrichment medium because it has been widely used and recommended for this purpose and because liver was readily obtainable and the medium easily prepared. Thioglycollate medium, which was employed for toxin production, was prepared from the dehydrated form manufactured by the Baltimore Biological Laboratory.

Sterile, 25 x 200 mm., cotton-stoppered, Pyrex culture tubes were used for collection of soil samples. Soil samples were obtained from personal acquaintances of the writers, from county farm advisers, and from members of the Department of Agronomy of the University of Illinois. Persons who collected samples were supplied with sterile, cotton-stoppered culture tubes packed in a mailing tube. Each individual was instructed to fill the tubes with surface soil from various sources, such as gardens, cultivated fields, roadsides, pastures, or barn lots.

Care was taken to prevent contamination of soil samples in the laboratory. They were held in culture tube racks in a room separate from the laboratory used for other culture work and were handled with the same precautions with which any bacteriological culture is handled.

Toxicity tests of cultures were made by feeding one ml. of each culture to a 250-gm. guinea pig. At the beginning of the work, only one culture was fed to an animal and the animal was observed for four days after the feeding. So few toxic cultures were found with each feeding that it was

decided to composite cultures and feed an aliquot of such size that an animal received one ml. of each culture. At the same time the period of observation of animals was increased to seven days. Three enrichment cultures represented the maximum number fed to any one animal. Only enrichment cultures from the same county were composited. When death of an animal occurred, the cultures were refed singly to individual animals to determine which cultures were toxic. In addition to being more economical both in time and labor, this method had the advantage of serving as a recheck on the toxicity of cultures.

Aside from prolonged periods of sterilization, incubation of media before use, and meticulous care in handling soil samples, other precautions were taken to prevent laboratory contamination.

Both before and after cultures were transferred, laboratory desks were flooded with a 1-1000 aqueous mercuric chloride solution. The area of the desk where drops of cultures might fall during pipetting was covered with cotton saturated with mercuric chloride solution. Occasionally the floor was wiped with mercuric chloride solution.

Desk tops were washed with mercuric chloride before soil samples were weighed. The balance was placed on a towel kept moist with the disinfectant. Soil particles falling from the spatula or weighing tube were caught in a dish of mercuric chloride solution.

All glassware, most of which was new, was sterilized before use by autoclaving two or three hours under 20 pounds pressure or in the hot-air oven at 121.1°C.(250°F.) for the same period of time.

New 250-gm. guinea pigs were used for typing experiments. Two ml. each of *Clostridium botulinum* antitoxin type A, type B, and type C were given to individual animals by injection into the peritoneal cavity. In early typing experiments these animals and a fourth untreated animal which served as a control were fed one to four ml. of the culture to be typed. Considerable difficulty was encountered in attempting to type cultures by this method and therefore in subsequent typing experiments, enrichment cultures were filtered and two to three ml. of sterile filtrate were injected subcutaneously. The latter procedure gave more consistent results.

RESULTS

A total of 604 soil samples from 88 of the 102 counties in the state of Illinois was examined; samples from which toxic cultures were obtained are listed (Table 1). The source, location, and time required for each culture to produce death are also indicated in the table.

If one considers each toxic composite as representing one toxic culture, then 44 out of 604 soil samples, or 7.2 per cent of the samples, formed toxic cultures. Toxin-producing samples were distributed among 26, or 25.4 per cent, of the counties in Illinois. The geographical distribution of toxin-producing samples indicates that the majority of such samples was obtained from the southern half of the state.

Omitting from consideration St. Clair county, which will be discussed individually, 34, or 12.1 per cent, of 279 samples examined from the southern half of the state produced toxic cultures. These samples were collected from 19, or 36.5 per cent, of the 52 counties in the southern part of the

TABLE 1

Source and Location of Illinois Soil Samples From Which Toxic Cultures Were Obtained

No.	County	Death time	Source	Location
		days		
1	Champaign	5½	Grass plot	Tolono
2	Christian	4	Soybean field	Edinburg
3	Clark	2½	Cornfield	Casey, Casey Twp., Sect. 21
4	Clark	36(hr.)	Garden	Marshal, Wabash Twp. Sect. 28
5	Clark	36(hr.)	Garden	City of Marshal
6	Clark	3½	Farm	Marshal Twp., Sect. 29
7	Clay	3	Plowed field	Neoga
8	Clinton	30(hr.)	.....	.....
9	Cook	2½	Yard	Beverly Hills
10	Cook	1½	Yard	Forest Park
11[1]	Cook	2	Lawn	Chicago
12	DeWitt	3	Composite of	
			soybean field	1 mi. N. W. Farmer City
			cornfield	1 mi. E. Farmer City
			chicken yard	1 mi. N. Kenny
13[1]	Douglas	12(hr.)	Plowed field	Dora
14[1]	Edwards	36(hr.)	Agron. expt. plot	West Salem
15	Jefferson	2½	Composite of	
			garden	Woodlawn
			yard	Woodlawn
			barn	Woodlawn
16	Jo Daviess	2½	Composite of	
			pasture	N. W. Stockton
			cornfield	S. W. Stockton
17	Johnson	36(hr.)	.....	1 mi. E. Simpson
18[1]	Kankakee	2	Peony garden	Kankakee
19	Macon	1½	Plowed field	1 mi. S. Oreana
20	Macon	4	Plowed field	5 mi. S. Decatur
21	Macon	5½	Plowed field	3 mi. S. Boody
22	Macoupin	12(hr.)	Strawberry garden	Benld
23	Macoupin	5	Pasture	Benld
24	Macoupin	4	Cornfield	Benld
25	Macoupin	3½	Brick yard	Benld
26	Madison	3½	Plowed field	Route 66, Collinsville
27[1]	Monroe	3½	Green	Waterloo
28	Montgomery	3½	Garden	Nokomis
29	Piatt	3½	Plowed field	Between Monticello and Cisco
30[1]	Piatt	22(hr.)	Plowed field	Between Monticello and Cisco
31	Pope	2	Turkey range	Robbs
32	Pope	15(hr.)	Garden	Robbs
33[1]	Rock Island	36(hr.)	Cornfield	¼ mi. N. Edgington
34	St. Clair	3	City yard	E. St. Louis
35[1]	St. Clair	1½	Agron. expt. plot	Lebanon
36	St. Clair	2½	Plowed field or roadside	Between Millstadt and Centerville
37	St. Clair	2	Plowed field or roadside	Between Millstadt and Centerville
38[1]	St. Clair	36(hr.)	.....	8 mi. W. Belleville
39[1]	St. Clair	36(hr.)	.....	11 mi. S. W. Belleville
40	Sangamon	3½	Bottom floodland	Illiopolis
41	Wabash	4	.....	.....
42	Wayne	5	Plowed field	Fairfield
43	Wayne	3½	Plowed field	Fairfield
44	White	3	Plowed field	Enfield

[1] The serological type of these cultures was determined.

state. These figures are quite in contrast to those for the northern part of the state where 10, or 3.65 per cent, of 274 samples were toxic and were obtained from seven, or 14 per cent, of the 50 counties in this half of Illinois.

As it was possible to secure a large number of widely distributed soil samples from St. Clair county, an intensive survey was made of this limited area. Fifty-one soil samples were examined in this county representing a ratio of one sample to every 13 square miles of area. Six samples, or 11.8 per cent, of the total samples from St. Clair county produced toxic cultures.

The serological type of 10, or 22.7 per cent, of the toxin-producing cultures was determined and all were found to form type B toxin.

DISCUSSION

Limitations of the technique for detection of *Clostridium botulinum* in soil are exceedingly great and it must be recognized that results can only be considered as indicative of the minimal distribution of the organisms.

It is interesting to note that with the exception of samples from Champaign, DeWitt, Douglas, Macon, and Piatt counties, which are located along the southern moraines of the Wisconsin glacial period, the toxin-producing samples were from areas of either sand, lake swamps, bottomlands, deep loess deposits and non-glaciated areas, or the large area glaciated during the earlier Illinoisian period, where in general the yellow and agriculturally less-productive soils of Illinois are found. With the exceptions noted above, the region subjected to Wisconsin glaciation, which created the most valuable agricultural lands in Illinois, was found free of toxin-producing soil samples. The possibility that physical-chemical characteristics and microbiological relations of the fertile black soils of northern Illinois may preclude detection of *Clostridium botulinum* by the techniques used, should not be overlooked.

Although the figure of 7.2 per cent for toxin-producing soils in Illinois is lower than that of 18.1 per cent obtained by Tanner and Dack (1922) and 12.8 per cent observed by Meyer and Dubovsky (1922b), the figures of 11.8 per cent for St. Clair county and 12.1 per cent for counties of southern Illinois, as obtained in this investigation, compare more favorably with results of these investigators.

The low figures for the occurrence of *Clostridium botulinum* in Illinois soils are confirmed further by the epidemiological history in which no cases of human botulism have occurred from home-canned food packed in Illinois and by the fact that there has been a low incidence of botulism among migratory water fowl which frequent the swamps and lakes of Illinois. However, rather than depreciating the hazard of botulism in Illinois, these figures confirm that botulism is a possibility and the danger is more widespread than previous investigators have shown.

The use of enrichment cultures inoculated with unheated soil in conjunction with isolations from shake cultures prepared from such enrichment cultures was an innovation introduced in this investigation for reasons to be discussed. Antibiosis is the primary limiting factor during attempts to reveal a maximum number of toxin-producing samples

by techniques available. This problem was partially circumvented by the use of a combination of methods in the hope that if one procedure failed to reveal a toxin-producing sample, the other would detect it. There were also further reasons. It is possible that in some cases *Clostridium botulinum* may exist in a vegetative state rather than in the spore form and thus would be destroyed by heating procedures. It was conceivable that this might be the case in soils where environmental conditions are conducive to a high degree of microbiological activity. Also it was believed this technique might reveal some less heat-resistant spores which could not be detected by other means.

The descriptions of colony appearance of *Clostridium botulinum* in agar shake cultures given by Dubovsky and Meyer (1922a) and Burke 1919b) were used as criteria for selecting colonies typical of *Clostridium botulinum* in this investigation. The disc-shaped colonies which they described were picked to the exclusion of all others except in cultures which had no such colonies, in which case colonies were chosen at random. Agar shake cultures were made of toxic cultures to obtain an idea of colony form of strains of *Clostridium botulinum* isolated from Illinois soils. Almost without exception the cultures presented the colony form described by Graham and Schwarze (1921), Bengston (1924), and Seddon (1922). Evidently cultures of *Clostridium botulinum* were obtained only from those shake cultures where the colonies were selected at random. Undoubtedly many cultures were missed because of this policy of picking only disc-like colonies, in preference to fluffy, ball-like colonies.

Original intentions to confirm all deaths produced on initial feeding of cultures by a second feeding were abandoned soon after the investigation was in progress because of difficulties encountered in replenishing the animal colony and in view of plans to type cultures at the end of the investigation. However, this was accomplished in effect when the change to feeding of culture composites was made later. Even with such confirmation the question of the toxicity of some cultures is not answered. There is little doubt as to the toxicity of cultures which produce death within 72 hours as borne out by a second feeding. However, in the period beyond this, representing a range of 96 to 144 hours, toxicity of cultures is questionable and confirmative feedings help little as they too may yield variable results.

Considerable difficulty was encountered during attempts to identify toxic cultures by antitoxin tests. Several investigators including Meyer and Dubovsky (1922b), Tanner and Dack (1922), and Damon and Payabal (1926) have reported similar difficulties. As most cultures were not typed until many of them were several months old, some had lost their toxicity and even subcultures of many failed to form toxin. It was found that more consistent results were obtained when the toxin was injected subcutaneously, rather than when it was fed to guinea pigs, and when new enrichment cultures were prepared from original soil samples in cases where the culture or subculture had lost its toxicity. Later in this investigation toxin-identification tests were made concurrently with toxin-production tests and this latter procedure is to be recommended most highly.

SUMMARY

Seven and two-tenths per cent of 604 soil samples examined from 88 counties in Illinois produced toxic cultures.

In the limited area of St. Clair county 11.8 per cent of the soil samples were found to produce toxin.

Twelve and one-tenth per cent of the soil samples from southern counties and 3.65 per cent of the samples from northern counties in Illinois produced toxic cultures.

Toxic cultures were obtained from soils of 25.4 per cent of the counties in Illinois.

Toxin-producing soil samples were obtained from 36.5 per cent of the southern counties and 14 per cent of the northern counties of Illinois.

These figures represent the minimal incidence of *Clostridium botulinum* in Illinois.

Ten, or 22.7 per cent, of the toxic cultures were identified serologically and all were found to be type B.

There appears to be a greater incidence of *Clostridium botulinum* in the geologically and agriculturally older and less-productive soils of southern Illinois than in soils of the northern portions of the state.

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EFFECTS OF DIFFERENT METHODS OF COOKING ON THE ASCORBIC ACID CONTENT OF CABBAGE[1]

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Cabbage, even when cooked, is generally considered a good source of ascorbic acid, but very little work has been done to compare the various common methods of cooking as to their relative efficiency in retaining this vitamin. Brinkman, Halliday, Hinman, and Hamner (1942) reported that with coarsely shredded samples, cooking in a tightly covered pan retained in the solid portion an average of 61, in a pressure saucepan 53, and in an open kettle 40 per cent of the ascorbic acid originally present in the raw sample. Wellington and Tressler (1938), on the other hand, report retentions of only 24 and 22 per cent when finely shredded cabbage was steamed or boiled, and 58 and 38 per cent when quartered cabbage was steamed and boiled. Olliver (1941) agreed in general with the last figures, for she obtained retentions of 52 and 35 per cent when quartered cabbage was steamed and boiled, respectively.

The present paper reports data from which comparisons among the following cooking methods can be made: boiling in an open and (to a limited extent) in a loosely covered pan, cooking in a tightly covered pan, in a pressure saucepan, and steaming.

EXPERIMENTAL PROCEDURES

Varieties and Sources of Cabbage: Three varieties of cabbage were used in the present study: Marion Market, Danish Ballhead, and Wisconsin Ballhead Yellow Resistant. The first two were grown in 1942 and 1943 under the supervision of Dr. A. E. Hutchins of the Horticulture Division on the University experimental plots. They were gathered at optimum maturity, and a generous number of heads, selected at random from a much larger number gathered at the same time, were sent to the laboratory for analysis. As many "gatherings" (hereafter referred to as "lots") were prepared and analyzed as could be handled during the stage of optimum maturity. The Wisconsin Ballhead cabbage was grown adjacent to the University experimental plots and was obtained through commercial channels.

Preparation and Cooking of Samples: The heads of cabbage were matched as nearly as possible according to size and general appearance and distributed to form two or three similar groups. All except one of the groups were placed in a refrigerator at 5°C.(41°F.). That group was used for preliminary cookings which were made to find the exact cooking periods necessary for the particular lot of cabbage being handled. As soon as the preliminary cookings were over, the heads belonging to another group

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were removed from the refrigerator and cut into as many pie-shaped sections as could be cooked and prepared for analysis within the next two hours (fourths for the 1942 lots, sixths for the 1943 ones). One pie-shaped section from each head was assigned to the raw and to each of the cooking methods, except in the case of the Wisconsin Ballhead, when sections were assigned to the raw and triplicate cookings by the same method. The sections intended for cooking were wrapped in waxed paper and returned to the refrigerator. Those in the raw sample were coarsely shredded at once, the shredded material was well-mixed, a 20-gram portion was weighed out and dropped into a Waring blendor containing 100 ml. of metaphosphoric acid. The samples reserved for cooking were withdrawn from the refrigerator, one after the other, as quickly as they could be handled, were coarsely shredded, mixed, a 215-gram and a 25-gram portion weighed, the latter tied into a loose cheesecloth bag, and both cooked together by the predetermined method for the length of time decided upon during the preliminary cooking tests (Table 1). The vegetable was then drained, the contents of the bag emptied immediately into a Waring blendor containing 100 ml. of cold, three-per cent metaphosphoric acid, and an aliquot of the cooking water set in an ice bath. The procedure of bagging the ascorbic acid sample bases the ascorbic acid determination directly on raw weight and eliminates the cooling period (and the possibility of oxidation during that time) which is necessary if small cooked portions are to be weighed with even a moderate degree of accuracy.

The third group of heads was used on the following day. In 1942 it was used to complete the series of cookings, since there were too few workers to do so in one day; in 1943, it was used, whenever available, to repeat the first day's work. In either case, the general treatment was the same as outlined, including analysis of the raw cabbage.

As previously indicated, 240 grams of cabbage were cooked by each method. The cooking methods used in both 1942 and 1943 were as follows: (1) boiling in an uncovered pan in 960 ml. of tap water—an amount which left just enough (650 to 750 ml.) to cover the vegetable at the end of the cooking period; (2) cooking on a rack one-eighth inch deep in a tightly covered pan containing 60 to 90 ml. of tap water— 60 for all the Marion Market samples except Lot 4, 90 for all other samples—of which only 20 ml. or less were left at the end of the cooking period; (3) cooking on a rack one-quarter inch deep in a pressure saucepan containing 60 ml. of tap water (about 30 ml. of which was usually left at the end of the cooking period); and (4) steaming. In addition, in 1942 the following methods were used: boiling in a loosely covered pan in one-half the water used in the uncovered pan, and cooking in a tightly covered pan in 30 ml. of water for Marion Market and 60 ml. for Danish Ballhead and Wisconsin Ballhead samples.

Methods of Analysis: The ascorbic acid determinations were made by the Morell method (1941), except that the correction of Bessey (1938) for color and turbidity was retained. Moisture was determined by heating 10-gram samples in flat, aluminum weighing dishes at 95°C.(203°F.) at 25 inches of vacuum for 18 hours.

TABLE 1

Cooking Periods for Three Varieties of Cabbage[1] Cooked by Various Methods

Variety	Year	Lot No.	Cooking period					
			Open kettle (960 ml. water)	Loosely covered kettle (480 ml. water)	Tightly covered kettle		Pressure saucepan (60 ml. water)	Steamer
					Minimum water (30-60 ml.[2])	Slightly larger than minimum (60-90 ml.[2])		
			min.	*min.*	*min.*	*min.*	*min.*	*min.*
Marion Market	1943	1(a,b)	4.5	....	....	15	3.0	27
	1943	2(a,b)	4.5	....	....	15	3.0	27
	1943	3(a,b)	9.0	....	....	25	5.0	40
	1943	4	12.0	....	....	40	8.5	50
	1942	1	4.0	4.5	11	11	1.5	22
	1942	2	3.5	3.5	11	11	1.5	20
Danish Ballhead	1943	1	10.0	....	....	35	7.0	40
	1943	2	12.5	....	....	40	8.5	50
	1943	3	12.5	....	....	40	9.0	50
	1943	4	12.5	....	....	40	8.5	50
	1942	1	8.0	8.0	16	16	2.0	24
Wisconsin Ballhead Yellow Resistant	1942	....	7.0	8.0	15	15	3.0	12
	1942	....	7.0	8.0	15	15	3.0	12
	1942	....	7.0	8.0	15	15	3.0	12

[1] 240 grams of cabbage were used for each cooking. [2] The smaller quantity was used for all of the Marion Market samples except Lot 4, the larger quantity for all other samples.

The minimum level of significance between means at the five-per cent point was calculated as follows:

$$\text{Minimum level of significance at five-per cent point} = t \times \sqrt{\frac{\text{mean square for error} \times 2}{\text{number of replications}}}$$

in which the mean square for error was taken from an analysis of variance in which cookings and replications were the sources of variation; and t equals the "t" value at the five-per cent point for the number of degrees of freedom of error.

DISCUSSION OF RESULTS

Figures for the ascorbic acid content of the raw and cooked cabbage (Table 2) are of particular interest to those planning dietaries, but the ones for the raw samples are also of general interest. The latter show that storage in the head at 5°C. for approximately 24 hours did not affect the ascorbic acid content, as is evidenced by the nonsignificance of Fisher's "t" value. These findings are in general agreement with those of Gould, Tressler, and King (1936), who reported that Glory variety of cabbage stored for 19 days at 1 to 3°C.(33.8 to 37.4°F.) retained all of its original ascorbic acid.

All of the raw samples of both varieties compare rather unfavorably in ascorbic acid content with the various raw samples of cabbage reported in the literature, but after cooking are quite like the few similarly prepared ones which are reported. Thus the raw portions, as may be seen (Table 1), ranged between 28.5 and 54.8 mg. per 100 grams fresh weight and therefore fall in the lower third of the range (24 to 181 mg. per 100 grams) displayed by the different samples summarized by Booher, Hartzler, and Hewston (1942). The comparison is no more favorable if made on the basis of variety, for both contain only about one-fourth as much ascorbic acid as samples of the same variety grown in Ohio on silty clay loam soil, fertilized with N:K:P::20:50:21, and the Danish Ballhead only about two-thirds as much as the average of 15 Montana samples—Mayfield and Richardson (1940). The latter variety, however, did outrank, by about 50 per cent, heads grown on New York clay loam soil — Gould, Tressler, and King (1936). The portions cooked in the tightly covered kettle, the pressure saucepan, and the steamer contained about the same amount of ascorbic acid as similarly prepared samples reported by Brinkman, Halliday, Hinman, and Hamner (1942) and by Wellington and Tressler (1938); the boiled samples were close to the average reported by Brinkman *et al.* (1942), but were considerably lower than that reported by Mayfield and Richardson (1940) for the Danish Ballhead variety. It should be pointed out, however, that agreement among cooked samples may not be maintained to this extent when further work is reported, for at present all of the samples for which there is sufficient cooking data to permit comparison have originally contained less than 65 mg. of ascorbic acid per 100 grams of vegetable.

The foregoing comparisons have been made on the basis of moist weight because moisture content was not available in all cases. The general conclusions would be the same on the dry basis, however, if the difference in moisture content among the various samples was no greater than between

TABLE 2

Ascorbic Acid Content of Raw and Cooked Cabbage

Variety	Year	Lot No.[1]	Time between gathering and sampling[2]	Raw (mg. per 100 grams)		Cooked (mg. per quantity equivalent to 100 gm. raw fresh wt.)					
				Dry wt.	Fresh wt.	Open kettle	Loosely covered kettle	Tightly covered kettle		Pressure saucepan	Steamer
								Minimum water	Slightly larger than minimum		
			hr.								
Marion Market	1943	1a	0-5	491	32.3	12.4	.....	.....	26.3	23.0	22.7
	1943	1b	24-27	445	28.5	15.4	.....	.....	30.6	26.5	25.0
	1943	2a	0-4	625	40.6	14.3	.....	.....	27.9	27.8	28.5
	1943	2b	24-27	555	37.2	12.3	.....	.....	27.1	22.3	21.0
	1943	3a	0-4	576	36.9	9.4	.....	.....	22.9	20.5	21.4
	1943	3b	24-27	597	44.2	13.6	.....	.....	27.0	26.7	12.7
	1943	4a	0-2	497	33.8	9.0	.....	.....	24.7	16.5	28.8
	1942	1a	30-36	.....	41.2	17.9	20.6	.....	.....	31.0	.....
	1942	1b	50-56	.....	37.8	.....	.....	34.4	33.6	.....	.....
	1942	2a	26-30	.....	38.6	25.1	23.4	.....	.....	42.2	.....
	1942	2b	50-54	.....	44.2	.....	.....	37.5	34.8	.....	33.4
Mean	1943	....	....	541	36.2	12.3	.....	.....	26.6	23.3	22.9
Mean	1942	....	....	.....	40.4	21.5	22.0	36.0	34.2	36.6	33.4
Danish Ballhead	1943	1	4-8	793	54.8	17.9	.....	.....	21.4	23.4	23.1
	1943	2	4-8	635	47.6	13.4	.....	.....	21.4	24.2	24.1
	1943	3	4-7	571	41.7	11.7	.....	.....	21.1	14.6	23.7
	1943	4	0-2	566	43.6	12.5	.....	.....	18.1	20.2	21.8
	1942	1a	4	.....	42.2	21.1	32.1	.....	.....	35.5	.....
	1942	1b	29	.....	47.6	.....	.....	41.1	33.8	.....	36.1
Mean	1943	....	....	639	46.9	13.9	.....	.....	20.5	20.6	23.2
Mean	1942	....	....	.....	44.9	.....	.....	.....	.....	.....	.....

[1] The letters "a" and "b" which follow the lot number indicate that the samples were taken from the same gathering. [2] Vegetables were stored at 5°C. (41°F.) during this period.

the 1943 ones reported in this paper (an average of 93 per cent for the raw, 92 per cent for the cooked) and those of Mayfield and Richardson (1940) (90 per cent raw, 95 per cent cooked).

The percentage retentions of ascorbic acid in the cabbage samples during cooking (Table 3), calculated from the data in Table 2, show considerable variation among the different lots within each of the methods of cooking. This variation cannot be accounted for by differences in the cooking time, as may be seen by comparing the cooking periods (Table 1) and the retentions (Table 3). Both varieties, for example, showed only a slight decrease in ascorbic acid retention when cooked in the open kettle for 12 as against four minutes (Marion Market variety) or 10 against 12.5 (Danish Ballhead variety). In the other cooking methods the retention did not change consistently with the cooking period.

In order to gain some information as to how much of this variation might be due to uncontrolled factors within the method of cooking, a series of cookings were made by the same method on replicate samples. In other words, the individual samples were prepared as previously described but were distributed among the raw and triplicate cookings by the same method rather than among the raw and different cooking methods. As explained previously, commercial samples of Wisconsin Ballhead Yellow Resistant cabbage, which was grown adjacent to the University plots, were used for this series of determinations; results are given (Table 2). The average deviations from the means were $\pm$ 2 to 3 for four of the methods, $\pm$ 0.3 for one, and $\pm$ 5 for the other. These deviations are practically the same as those of Brinkman *et al.* (1942). If similar conditions held for the Marion Market and Danish Ballhead varieties, it would seem therefore that all of the difference within the cookings by the open-kettle method can be accounted for by uncontrolled factors in the cooking method, but that part of the variation within each of the other methods must be due to some other factor as yet unidentified.

The relative effectiveness of the different cooking methods in retaining ascorbic acid is shown (Table 3). In order to facilitate this comparison, the minimum level of significance between the means was calculated by the method outlined previously and has been designated in the table by the letters MLS. According to this criterion the pressure saucepan, the tightly covered pan, and the steamer, it will be noted, were equally effective for the 1943 samples in retaining ascorbic acid; also, all three were more effective than cooking in the open kettle. For the 1942 Marion Market samples, on the other hand, all of the methods were equally effective; here, however, the minimum level of significance was very high, owing to the wide variation between lots and to the fact that only two lots were used. For the Wisconsin Ballhead the averages for the tightly and loosely covered kettles and for the steamer were not significantly different from one another, but that for the pressure saucepan fell below them, while that for the open kettle was again the lowest of all. Thus, while there is some disagreement, the bulk of the present evidence indicates that the tightly covered vessel, the steamer, and probably the pressure saucepan retain, on the average, approximately the same proportion of the original ascorbic acid during cooking. The amount is significantly larger than

[1] The letters "a," and "b," which follow the lot number indicate that the samples were taken from the same gathering. [illegible] vegetables were stored at 5 C. (41 F.) during this period.

TABLE 3

Retention of Ascorbic Acid in Cabbage During Cooking by Different Methods

Variety	Year	Lot No.[1] and MLS[2]	Ascorbic acid retained in vegetable						Ascorbic acid dissolved in cooking water					
			Open kettle	Loosely covered kettle	Tightly covered kettle		Pressure saucepan	Steamer	Open kettle	Loosely covered kettle	Tightly covered kettle		Pressure saucepan	Steamer
					Minimum water	Sl. larger than min.					Minimum water	Sl. larger than min.		
			pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Marion Market	1943	1a	38	....	....	80	70	69	48	....	....	3	4	4
	1943	1b	46	....	....	90	78	74	48	....	....	5	2	3
	1943	2a	35	....	....	69	68	70	42	....	....	1	6	4
	1943	2b	33	....	....	73	60	56	45	....	....	2	2	1
	1943	3a	26	....	....	62	56	58	51	....	....	0	4	9
	1943	3b	32	....	....	61	60	29	50	....	....	2	1	0
	1943	4	27	....	....	73	49	85	56	....	....	7	6	0
	1942	1	43	50	91	89	75	....	43	35	1	2	5	....
	1942	2	65	61	85	79	109	76	41	32	0	4	2	2
Mean	1943	MLS: 10	34	....	....	73	63	66	49	....	....	3	4	3
Mean	1942	MLS: 36	54	55	88	84	....[3]	76	42	34	1	3	4	2
Danish Ballhead	1943	1	33	....	....	39	43	42	29	....	....	3	5	1
	1943	2	28	....	....	45	51	51	55	....	....	3	2	3
	1943	3	28	....	....	51	35	57	53	....	....	3	5	4
	1943	4	29	....	....	42	46	50	54	....	....	4	5	4
	1942	1	50	76	86	71	84	76	43	32	5	10	6	2
Mean	1943	MLS: 9	30	....	....	44	44	50	48	....	....	3	4	3

[1] For meaning of "a" and "b" attached to the lot number, see Table 2. [2] Minimum level of significance at five-per cent point = t x standard error of the difference between two means. [3] Average was not calculated because of the apparent gain in Lot 2.

when the open-kettle method of cooking is used. It should be kept in mind, however, that occasionally an individual lot does not follow this order—for example, Marion Market Lot 3b, in which the steamed sample retained less ascorbic acid than the one cooked in the open kettle.

The percentage of ascorbic acid dissolved in the cooking water at the end of the cooking period is also given (Table 3). As will be noted, the quantity in the water from the tightly covered pan, the pressure saucepan, and the steamer contained an insignificant proportion of the ascorbic acid originally present in the raw sample, since the average for each method was less than five per cent. The amount in the cooking water from the open kettle was large, however, usually being between 45 and 55 per cent of that present in the raw sample.

Because of too few workers, measurements to determine the relative quality of the samples cooked by the different methods were not attempted. Observations indicated, however, that the cabbage cooked in the open kettle was always creamy white and bright green in color and possessed a mild, cabbage-like odor, while the samples cooked in the tightly covered pan and in the steamer were greyish-white and yellow-green in color with a strong odor that no longer reminded one of the original vegetable. The samples cooked in the pressure saucepan were similar to the boiled ones when the cooking time was short, but they were like the tightly covered or steamed ones if the cooking period extended much beyond three minutes.

SUMMARY

Four lots of Marion Market and a similar number of Danish Ballhead cabbage, freshly gathered at various times during the 1943 growing season, averaged, respectively, 36.2 and 46.9 mg. ascorbic acid per 100 grams of vegetable; while two lots of Marion Market and one of Danish Ballhead gathered during 1942 averaged 40.4 and 44.9 mg. per 100 grams.

There was considerable variation in the percentage retention of ascorbic acid within each of the methods of cooking. The variation could not be accounted for by differences in cooking time, and for only the open-kettle method did it seem to be wholly due to uncontrolled factors within the method of cooking.

The minimum level of significance between means was calculated, and according to this criterion, the bulk of the data indicated that the steamer, the tightly covered pan, and probably the pressure saucepan were equally efficient in retaining ascorbic acid, while the open-kettle method of cooking retained a significantly smaller proportion; occasionally, however, an individual lot did not follow this order. The former methods showed an average retention in 1943 (when the majority of the samples were cooked and analyzed) of 67 and 46 per cent for Marion Market and Danish Ballhead cabbage, respectively, and the open-kettle method an average of 34 and 30 per cent.

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ASCORBIC ACID CONTENT OF OKRA AS AFFECTED BY MATURITY, STORAGE, AND COOKING

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The ascorbic acid content of okra (*Abelmoschus esculentus*, L.) is of importance in determining the vitamin C value of the southern dietary because okra grows through the hot summer months when few fresh garden vegetables are available. The only information found in the literature is that reported by Burrell and Ebright (1940), who found that two samples of the Long Green variety contained 0.147 milligram of ascorbic acid per gram of fresh weight.

The purpose of this study was to obtain more information concerning the ascorbic acid content of okra and the effects of some selected factors upon this content—namely, maturity, storage, and various methods of cooking.

EXPERIMENTAL PROCEDURE

Two very similar varieties of okra, Louisiana Green Velvet and Louisiana White Velvet, were used in the present investigation. During the months of July and August, Louisiana Green Velvet okra was harvested and used for the experimental work; during September, October, and November the Louisiana White Velvet variety was used. They were grown at the Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

Sampling: For studies of storage and cooking losses, pods from five to five and one-half inches in length were selected. At this stage of development the pods were of good eating quality and large yields were obtained. The okra was cultivated on a one-half acre plot of ground which was divided into 22 rows. All of the five to five and one-half inch pods were gathered before sunrise from the first two rows on the first day they were analyzed, the second two rows on the second day, and so on until the last two rows of okra had been gathered. The same order for gathering was repeated when all of the rows had yielded a portion of the sample. The pods were placed in a refrigerator for no longer than an hour and a half, removed immediately before analysis, and mixed thoroughly. A sample for analysis consisted of 10 pods which were cut crosswise into one-fourth to one-half inch slices, and mixed thoroughly to insure a representative sampl from all parts of the pods.

Method of Analysis: Since larger samples gave an extract which was too viscous to centrifuge well, 20-gram samples were used. The samples were ground in three-per cent metaphosphoric acid in a Waring blendor for three minutes and made up to a volume of 250 milliliters. After centrifuging, a suitable aliquot of the clear extract was titrated with sodium

2,6-dichlorobenzenoneindophenol solution which was standardized daily against pure ascorbic acid. All samples were analyzed in duplicate according to the above procedure, and the results of duplicate analyses were averaged.

Duplicate moisture determinations were made on all samples so that the ascorbic acid value could be calculated on the basis of the percentage of dry matter.

MATURITY STUDIES

The Louisiana Green Velvet okra analyzed was edible from the time the pods first began to develop until they reached a length of about six inches. The fully matured pods 10 to 10½ inches long were woody and undesirable. The small, immature pods contained large amounts of ascorbic acid (Table 1), and as the pods increased in length and maturity the ascorbic acid content decreased. This was true on both the moist- and dry-weight bases. The most immature pods and the most mature pods contained the highest percentages of dry matter.

TABLE 1

Effect of Maturity on Ascorbic Acid Content of Louisiana Green Velvet Okra [1]

Length of pod	Ascorbic acid content on moist-weight basis [2]		Dry matter		Ascorbic acid content on dry-weight basis	
	Av.	Range	Av.	Range	Av.	Range
in.	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*pct.*	*mg./gm.*	*mg./gm.*
2½ to 3	42	38-47	11.2	11.4-11.1	3.72	3.45-4.12
4 to 4½	32	28-36	9.8	9.5-10.0	3.29	2.83-3.76
5½ to 6	25	23-28	9.1	9.1- 9.1	2.76	2.55-3.04
10 to 10½	16	12-18	11.0	10.5-11.4	1.47	1.04-1.74

[1] Each figure is an average of the results of four determinations. [2] 5 to 5½-inch pods which were analyzed repeatedly during cooking and storage studies ranged from 28 to 15 milligrams of ascorbic acid per 100 grams of fresh weight.

STORAGE STUDIES

To determine the effect of storage upon ascorbic acid values, samples were analyzed immediately after harvesting, while similar samples gathered at the same time were placed in brown paper bags and stored at temperatures of 27.8 to 30°C. (82 to 86°F.) and 3.3 to 4.4°C. (38 to 40°F.) for 24 and 48 hours before analysis; the results of these analyses are reported (Table 2). At the end of 48 hours of storage at refrigerator temperature the okra pods still retained 92 per cent of the original ascorbic acid, while pods stored at room temperature retained less than 50 per cent.

COOKING STUDIES

Okra pods were cooked under eight varying experimental conditions, seven of which were judged by the authors to be the most commonly used methods of cooking. Steaming under pressure in a pressure saucepan was included in the methods because previous studies have shown that vegetables cooked in this manner retained a high percentage of ascorbic acid. All samples were cooked in a two-quart, heavy aluminum, uncovered sauce-

pan with the exception of those cooked in the pressure saucepan. Distilled water was used for cooking and for all solutions.

Boiling: Preliminary tests were made to determine minimum cooking time and minimum cooking liquid, which showed that a cooking time of 20 minutes was satisfactory when judged by 10 persons testing the product by tasting and cutting. Minimum cooking liquid is defined as that quantity of liquid which would be evaporated by the end of the cooking period.

TABLE 2

Ascorbic Acid Content of Okra as Affected by Storage at Room [1] and Refrigerator [2] Temperatures [3]

Method of storage	Moist weight				Dry weight		
	Before storage	After storage	Retention	Dry matter	Before storage	After storage	Retention
24 hours	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*pct.*	*mg./gm.*	*mg./gm.*	*pct.*
Room temp.	30	19	64	8.49	3.49	2.28	65
Refrig. temp.	24	24	100	9.02	2.93	2.77	94
48 hours							
Room temp.	23	11	46	11.62	2.56	0.93	36
Refrig. temp.	26	24	92	9.80	2.83	2.45	87

[1] 27.8 to 30°C.(82 to 85°F.). [2] 3.3 to 4.4°C.(38 to 40°F.). [3] Each figure is an average of the results of four determinations.

Five different methods of boiling were employed:

1. Whole pods from which the stem ends were removed were slowly added to a minimum amount (245 gm.) of rapidly boiling water so that the water into which they were dropped never stopped boiling. Toward the end of the 20-minute cooking period the pods were lifted occasionally to avoid sticking.

2. Sliced okra pods were boiled in the same manner as in Method 1.

3. Sliced okra pods were boiled as in Method 1, but for an extended time, 60 minutes.

4. Sliced okra pods were cooked in the same manner as in Method 1 with the exception that tomato juice was used as the cooking liquid. The pH of the okra and the tomatoes before cooking was 5.78 and 4.04, respectively. The pH of the combined okra and tomato juice after cooking was 4.20. The ascorbic acid value of the tomato juice as well as that of the okra was determined before cooking and considered in relation to the ascorbic acid value of the cooked product when calculating the percentage retention.

5. Whole pods were dropped slowly into an excess amount (500 gm.) of rapidly boiling water and boiled 20 minutes. At the end of the cooking period the pods and cooking water were poured on a wire sieve and allowed to drain for three minutes. Ascorbic acid determinations were made on the cooking water and on the pods.

Steaming Under Pressure: Whole pods were cooked at 115°C.(239°F.) for two minutes in a three-quart, stainless-steel pressure saucepan.

Preliminary Dry Heat: The sliced pods were placed in a hot aluminum saucepan and stirred constantly for five minutes, at which time the okra no longer appeared mucilaginous, and then boiled 15 minutes with minimum water.

Sautéing: Sliced pods were fried for 15 minutes with constant stirring in 11 grams of hot, hydrogenated vegetable fat.

The results from these cooking studies are shown (Fig. 1).

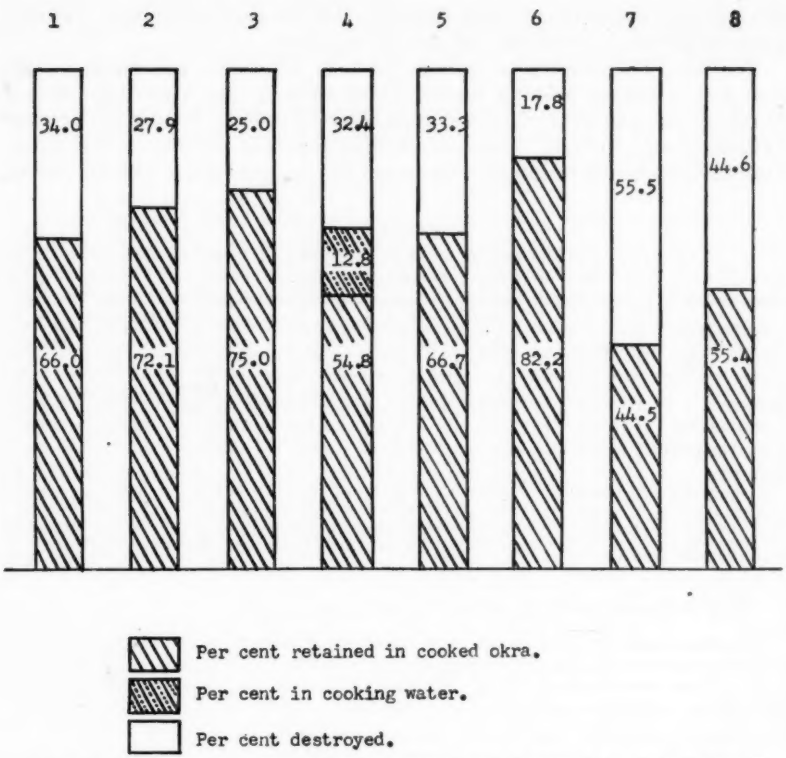

FIG. 1. Percentage of ascorbic acid retained and destroyed in cooked okra.

Explanation of bar graph (Fig. 1):

1. Whole pods boiled 20 minutes, minimum water.
2. Sliced pods boiled 20 minutes, minimum water.
3. Sliced pods boiled 20 minutes, minimum tomato juice.
4. Whole pods boiled 20 minutes, excess water.
5. Sliced pods boiled 60 minutes, minimum water.
6. Whole pods cooked two minutes at 115°C.(239°F.) in pressure saucepan.
7. Sliced pods cooked with dry heat five minutes, moist heat 15 minutes.
8. Sliced pods fried 15 minutes in small amount of fat.

(Each figure is an average of four determinations.)

SUMMARY

Young okra pods were found to be a good source of ascorbic acid and may be of value in the southern dietary because okra grows through the hot summer months when few fresh garden vegetables are available. The small, immature pods, two and a half to three inches long, contained an average of 42 milligrams of ascorbic acid per 100 grams of fresh weight. As the pods increased in maturity the ascorbic acid content decreased. Mature 10-inch pods of Louisiana Green Velvet okra contained an average of 16 milligrams of ascorbic acid per hundred grams of fresh weight.

Greater losses in ascorbic acid content occurred when okra was stored at room temperature than when stored at refrigerator temperature for 24 and 48 hours.

Okra cooked in the pressure saucepan retained the highest amount of ascorbic acid, 82.2 per cent. Retention of ascorbic acid by other methods ranged between 44.5 and 75 per cent. Rapid initial heating seemed to be of primary importance in ascorbic acid retention.

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BACTERIOLOGICAL STUDIES RELATING TO THERMAL PROCESSING OF CANNED MEATS[1]

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Evaluation of thermal processes for canned foods has been a subject of scientific research for some 25 years. The General Method, for calculation of thermal processes, described by Bigelow, Bohart, Richardson, and Ball (1920), was the first scientific approach to the problem of applying bacteriological and physical data to the evaluation and calculation of thermal processes. This method is still used in the canning industry, especially for evaluating processes for foods for which the heating curves cannot be represented by one or two straight lines — Schultz and Olson (1940). Simpler and more versatile methods for process calculation were developed by Ball (1923, 1928). Ball's methods have been used extensively in the canning industry. The simplicity and flexibility of the methods have contributed greatly toward the solution of many practical processing problems. Numerous simplifications and improvements in the above methods of process calculation and evaluation have been made by Schultz and Olson (1938, 1940), Olson and Stevens (1939), and Jackson and Olson (1940).

By the procedures for process evaluation now available, it is possible to assign relative sterilizing values to heat processes used in the manufacture of practically all canned foods. The F values, obtainable by mathematical treatment of physical and bacteriological data, express in simple numbers the relative effectiveness of different heat processes for canned foods. However, because of the great variation in thermal resistance of bacteria, it has not been possible to establish a generally applicable rule for ascertaining whether a given process will always, or even most of the time, prove satisfactory and desirable for a given product manufactured under conditions of practical operation.

The thermal resistance of any species of bacteria varies with many factors, one of the most important of which is the nature of the substrate in which the bacteria are subjected to heat. Among other factors which may influence the thermal resistance of any given species of bacteria are initial concentration of cells, presence or absence of heat-resistant spores, and age of bacteria. In meat, the picture is complicated by the fact that many different species of bacteria contaminate the product under conditions of practical operation.

If the rate at which heat penetrates a can of food and the nature of the thermal death time curve for a given organism in that food are known,

[1] In this and future papers only those meats which are hermetically sealed in the can and given heat processes designed to prevent their spoilage in the course of commercial handling without special treatment will be considered as canned meats.

[2] Present address: Michigan State Department of Health, Lansing, Michigan.

it is possible by methods referred to above to determine the minimum thermal process sufficient to sterilize that food, with respect to the given organism, only if the factors which govern the heat resistance of the organism remain constant. The rate of heat penetration can be readily determined for most canned foods. However, to determine the nature of a thermal death time curve requires considerable technical training, and the time involved for a thermal death time curve determination sufficiently accurate and complete to allow successful translation of results into practice is virtually prohibitive because of the numerous factors which influence the heat resistance of bacteria.

A brief description of some of the terms employed in the calculation and evaluation of thermal processes for canned foods will illustrate some of the difficulties involved in applying the results of such calculations and evaluations in canning practice. The following terms, or their equivalents, are involved in all fundamental procedures for arriving at thermal processes for canned foods.

z. A value which represents the slope of the thermal death time curve for an organism.

F. A numerical value equal to the number of minutes theoretically required to destroy an organism at 121.1° C. (250° F.), assuming instantaneous heating and cooling of the medium surrounding the organism.

The z value is relatively difficult to determine; and, because of the nature of canned meats, the value of z has commonly been assumed to be 18°F. for the bacteria ordinarily concerned in canned meat spoilage. To determine the value of z for a given organism in a given medium it is necessary to determine the number of minutes, at each of several lethal temperatures, required to destroy the organism in the given medium. The thermal death time curve is obtained by plotting on semi-log paper temperature in the direction of abscissae and time, required to kill the organism, in the direction of ordinates. The z value, referred to as the slope of the curve, is equal to the number of degrees Fahrenheit required for the curve to pass through one logarithmic cycle.

As pointed out by Ball (1928) no established rule can be given to govern the choice of a value of z. Ball states that the proper value depends on a large number of factors, one of the most important of which is acidity. Further, that for products having pH values between 5.6 and 6.4, the value of z is generally taken as 18°F. Olson and Stevens (1939) made the following statements with reference to the value of z:

"In general, the value of z may be considered constant for a given strain of bacterial spores in a given medium, or conversely, the value of z will be different for different strains of spores in the same medium or for the same strain in different media. At present, very little is known concerning the factors which cause variations in z value.

"Early investigations by Esty and Meyer (1922) established the 'ideal' thermal death-time curve slope as 18° for spores of *Clostridium botulinum* suspended in neutral phosphate solution. Since then, further determinations have tended to confirm this value of z for spores of *Cl. botulinum* and some related putrefactive anaerobes when suspended in neutral phosphate. In food juices, however, it would appear that the slope of the thermal death-time curve for those organisms may vary over a range of

about 5° or 6° on either side of the 'ideal' 18° and thus cause considerable change in values obtained by process calculations.

"In the absence of more reliable information the 'ideal' z value, 18°, has been quite generally used for evaluation and calculation of processes. The only exceptions have been in the cases of particular foods where determinations with a certain organism had shown some other value of z to be more applicable."

Since the pH values for most canned meats usually lie in the range pH 5.6 to 6.4, it may be that the use of 18°F. is a procedure sufficiently accurate for practical purposes. There is need for further study designed to establish the z value for bacteria in meat. It should be pointed out that when the z value is 18°F., the F value may be designated as Fo to describe the special condition, Ball (1928). Sterilizing values of processes for canned meats are usually referred to as Fo values, thus designating that z has been either determined or assumed to be 18°F.

The value of F (defined above) is less difficult to determine than is the value of z; however, it cannot be assumed to be constant except for a given set of conditions. It is an expression of thermal resistance of bacteria and, therefore, subject to variation with any factor which influences thermal resistance of bacteria. Methods have been devised for determining thermal resistance of bacteria under a given set of conditions. However, translation of the meaning of F values into meat-canning practice has been difficult because of the lack of information concerning the influence of variable operating conditions on the thermal resistance of bacteria in meat.

The American Can Company (1943) described a method developed in its Research Laboratories for determining thermal resistance by the use of a battery of small specially constructed retorts and specially designed "T.D.T." (thermal death time) cans. Known concentrations of bacteria or bacterial spores are placed in food, in buffer solutions, or in nutrient media distributed in the "T.D.T." cans. The containers are sealed and then processed in the small retorts at various temperatures for varying times. The times required to destroy a known number of the test bacteria at several different processing temperatures are determined by incubating and examining the cans for evidence of survival of the organisms.

The Research Laboratories of the National Canners' Association have employed a slightly different method for studying thermal resistance—Yesair and Cameron (1942). Meat, or some other substrate, is inoculated with spores of the test organism in a minimum volume of liquid. The test material, unless of such nature that it can be introduced with a pipette, is introduced into small glass tubes by means of an Alemite gun. After sealing the tubes in the flame of a blast-burner, they are processed in an oil bath at various temperatures for varying times. Thermal resistance of the test bacteria is determined in one of three ways: (1) by incubating the processed tubes, (2) by culturing the contents of the processed tubes into a medium favorable for the growth of the test organism, or (3) by simultaneously incubating one lot of tubes and culturing the contents of a duplicate lot.

In laboratory determinations of thermal death time it has been a common procedure to use 10,000 or more spores of a pure culture of bacteria per gram of test material. The test bacteria have often been grown under

conditions highly artificial relative to those existing in meat. In making thermal resistance tests, it has been a common practice to suspend spores of the test bacteria in phosphate buffer solutions. Thermal death time determinations are tedious and laborious even when made under such special conditions as these. Moreover, factors which influence heat resistance of bacteria very definitely limit the use of thermal death times, thus determined, in ascertaining thermal processes required in canning practice. Therefore, it has been common to judge by past experience what sterilizing, or F, value a process must have in order for it to be used successfully in canning any particular product.

From heat-penetration data, which can be readily obtained, and the F and z values chosen, or determined, it is a rather simple procedure to calculate a thermal process in terms of time and retort temperature. However, it has been difficult and often expensive to determine if the calculated process were adequate to prevent spoilage of a product manufactured under conditions of practical operation. A popular procedure for determining this has been to incubate, for a given time, cans of product given the calculated process, the process being considered sufficient if the product did not spoil during the incubation period used. By this procedure, there has been no way of accounting accurately for the influence of changes in operating conditions upon the thermal resistance of bacteria present in the product. To overcome this difficulty some meat packers have incubated a certain percentage of cans from each day's manufacture; however, the times used for incubation are frequently insufficient. Spoilage often occurs several weeks or months after the meats are canned. The reasons for such delayed spoilage are not well understood and should receive the immediate attention of research men in the meat-packing industry.

A convenient, inexpensive method is urgently needed for determining, by routine examination of meats to be canned, the heat processes necessary to prevent spoilage of canned meats. Further studies concerning the influence of various factors upon the thermal resistance and growth of bacteria in meat should aid materially in establishing heat processes which are adequate to prevent spoilage of canned meats and which allow meats to be heat processed with minimum loss of their organoleptic and nutritional qualities.

The methods which follow are now being used in studies concerning the influence of various factors, such as curing agents and concentration of spores, upon the thermal resistance and growth of a putrefactive anaerobe in heat-processed canned meats. Since the results of these studies will be published in papers to follow, the methods are presented here as they are being employed in these studies.

I. LABORATORY METHODS EMPLOYED FOR STUDYING FACTORS WHICH INFLUENCE THERMAL PROCESSES REQUIRED TO PREVENT BACTERIAL SPOILAGE OF CANNED-MEATS

Putrefactive, anaerobic, spore-bearing bacilli (members of the genus *Clostridium*) have most commonly been the cause of spoilage of canned meats, unless the causative organism gained access to the meats subsequent

to the heat processes. The organism chosen for studying the influence of various factors upon thermal resistance and growth of putrefactive anaerobes in meat was isolated from spoiled, cured, canned meat. Strain 9x was isolated in the Research Laboratories of the American Can Company and Strain S_2 in this laboratory. The two strains were found to be culturally and serologically identical, except with respect to fermentation of sucrose. Strain 9x fermented sucrose, while Strain S_2 has not fermented this sugar in any of several determinations. Both strains fermented the following sugars with the production of acid: glucose, maltose, fructose, sorbitol, trehalose, dextrin, and salicin. In addition, both strains fermented glycerol and starch.

According to the key for classification of anaerobes presented by Spray (1936) both strains belong to Group IV. In many other respects, including heat resistance, toxicity, and optimum temperature for growth, both strains were indistinguishable and therefore considered to be strains of the same species. The organism, both strains, grew and produced heat-resistant spores abundantly in common laboratory media and in cooked and uncooked meat at temperatures between 28 and 37°C.(82.4 and 98.6°F.), most rapid growth occurring at 37°C. Whole cultures of the actively growing organism were nontoxic for guinea pigs and rabbits. The heat resistance of the spores of the organism exceeded the highest resistance ever reported for spores of known strains of *Clostridium botulinum* and *Clostridium sporogenes*. This permitted study of it in pure culture in meats since, after inoculating fresh meats with its spores, the meats could be given heat processes sufficient to destroy all other bacteria present and thus leave it in pure culture. Hence, growth and thermal resistance of the organism have been studied in meats handled, up to the time the meats were canned, under conditions of routine packing-house operation.

MEDIUM USED FOR SPORE PRODUCTION

To study heat resistance and growth of spore-bearing bacteria in pure culture in meat or some other chosen substrate, it is desirable to produce spores of the test bacteria in mass volume. The concentration of spores in the suspension used must be sufficiently high to allow the maximum number of spores employed to be added to the test substrate without appreciably diluting the substrate. Further, it should be possible to reproduce comparable spore suspensions when the need arises. The medium chosen in this laboratory as being most applicable was prepared as follows:

Five grams of salt (NaCl), 10 grams of Difco peptone, five grams of Difco beef extract, and five grams of dextrose were dissolved in each liter of water to make a nutrient broth. This broth was then distributed in test tubes or small bottles and an equal volume of chopped fresh sheep brains added. (Only the gray portion of the brains was used.) The chopped brains were introduced beneath the surface of the broth by means of an Alemite grease gun fitted with a large artery pumping needle.[3] The reaction of the medium was controlled by adding a slight excess of calcium carbonate to each container before the medium was sterilized.

[3] Obtained from Griffith Laboratories, 1415 West 37th Street, Chicago, Illinois

Production of Inoculum: The brain medium was prepared in eight-ounce, screw-cap bottles containing a few glass beads. The caps were screwed down tightly and the bottles autoclaved 30 minutes at 15 pounds steam pressure. Immediately after cooling, the medium was inoculated with one ml. of an actively growing culture of the test organism. (Medium which had been stored any length of time after sterilization was heated 30 minutes in a steam bath before it was inoculated.) After six to eight days' incubation at 37°C., the bottles were removed from the incubator and shaken, mechanically, for 30 minutes. With the putrefactive anaerobes studied in this laboratory the brain, after six days' incubation, was sufficiently digested so that it emulsified completely in the shaking process. It should be pointed out that, for less proteolytic organisms, a clear broth medium might prove superior for producing spore suspensions.

The emulsion obtained by shaking the culture was filtered through sterile glass wool. The concentration of spores in the filtrate was sufficient to allow the filtrate to be used directly as the inoculum in all studies conducted.

Estimation of Spores in Inoculum: A dilution method for spore estimation was adopted after failing to obtain consistent results from Spray dish cultures. Serial decimal dilutions of the brain broth filtrate (spore suspension) were made in distilled water. From each dilution, over the appropriate range, 10 tubes of brain medium were inoculated with one ml. each. After inoculation, the tubes of brain medium were heated at 80°C.(176°F.) in a water bath for 20 minutes. The tubes were then incubated at 37°C. In some instances, duplicate lots of tubes have been incubated at 28°C. with similar results; however, a longer incubation period was required at 28°C. The tubes were incubated until the number of tubes positive for growth remained constant for at least 48 hours. The count was estimated from the number of positive tubes.

Counts obtained over a period of six months on one lot of spore suspension kept under refrigeration are as follows:

Date	Spores per ml. of inoculum
June 21, 1943	8,000,000
June 21, 1943	7,000,000
July 6, 1943	8,000,000
July 28, 1943	6,000,000
August 4, 1943	7,000,000
August 19, 1943	9,000,000
October 30, 1943	8,000,000
December 21, 1943	7,000,000
December 21, 1943	8,000,000

PREPARATION OF MEATS FOR THERMAL RESISTANCE AND GROWTH STUDIES

Trimmings consisting of fresh blade meat, butts, or picnics were used in all studies. The trimmings were ground through a ⅛-inch plate. The ground meat was then thoroughly mixed in a power mixer and portioned. The portions were variously treated with curing agents commonly used

in curing meat and inoculated with predetermined amounts of suspensions of spores of a putrefactive anaerobe. Each of the variously treated portions was then mixed under vacuum in a power mixer for 15 minutes. A given quantity of each lot of differently treated meat was distributed, in approximately two-gram amounts, in acid-cleaned, 10 x 75 mm., glass test tubes. An Alemite grease gun fitted with the large artery pumping needle described above (Fig. 1) was used to stuff the meat into the tubes. Care was exercised during the stuffing procedure to avoid entrapment of air in or around the meat in the tubes. After stuffing, the tubes were sealed and stored 24 hours at approximately 3.3°C.(38°F.), a temperature commonly recommended for storage of meats being cured for canning. Con-

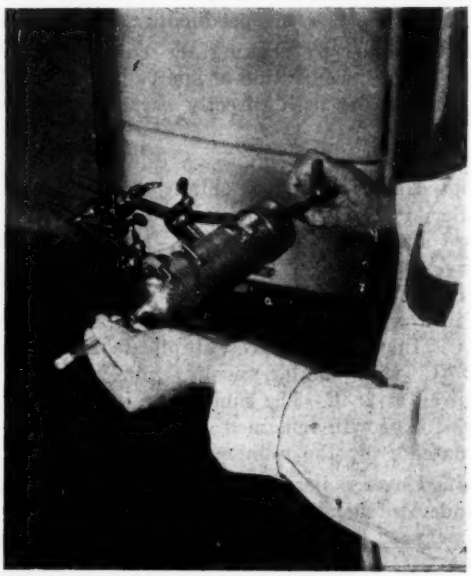

FIG. 1. Apparatus used for stuffing meat into tubes.

tamination of the meats was kept to a minimum by treating all equipment with a 0.5-per cent solution of sodium hypochlorite before and after it was used.

Sealing and Heat Processing Tubes of Meat: The tubes containing meat were sealed in the flame of a blast-burner. By bending a ring in the drawn-out glass before the tubes were sealed, it was possible to handle the tubes without tying them in compact bundles or crowding them in baskets (Fig. 2). The time involved in the sealing operation amounts to less than one minute per tube. It may be noted that small safety pins were used to fasten replicate tubes together.

A lard bath (Fig. 2) was used to process all tubes of meat and inoculated phosphate solution. A power stirrer was used in conjunction with the bath in order to assure uniform temperature distribution. The tem-

perature of the lard was controlled by a Dekhotinsky thermoregulator to a variation of ± 0.2°F.

Prior to processing, the tubes were all brought to a temperature of 79.4°C.(175°F.) in a second lard bath held at that temperature (five minutes being sufficient). The tubes were then transferred rapidly from the 175°F. bath to the process bath. After a predetermined number of minutes the tubes were removed from the process bath and immediately plunged into water at approximately 21.1°C.(70°F.). The process time was accurately kept with a stop watch.

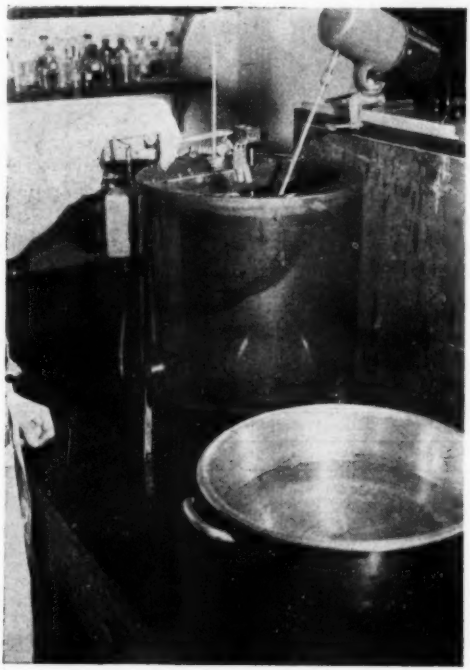

FIG. 2. Oil bath used for processing tubes of meat.

Evaluation of Heat Processes: Assuming the value of z to be 18°F., the theoretical process times, in minutes, necessary at each of four process temperatures to give Fo values from one to 16 were calculated according to the procedures presented by Ball (1928). It was established that curing mixtures to be added to the meat, in the course of the investigation, did not influence the slope of the thermal death time curve of the organism employed. Therefore, for purposes of comparison, F values corresponding to any value of z may be calculated from the time values given without altering the relative value of data obtained at any one processing temperature. Corrections, calculated from data upon the rate of heating and rate of cooling of meat in the tubes, were applied to the theoretical time

values to obtain the actual process times necessary. Rates of heating and cooling were measured with specially constructed thermocouples obtained from the Research Laboratories of the American Can Company. The procedures, employing special co-ordinate paper for thermal process calculations by the general method presented by Schultz and Olson (1940), were followed in ascertaining the corrections.

The time corrections determined for four different process temperatures are as follows:

Process temperatures	Corrections for meat
°F.	*min.*
220	1.93
230	2.05
240	2.18
250 (for Fo values above 1)	2.34

The correction for Fo value of 1 at 250°F. is 2.24.

It should be pointed out that these corrections were determined for processing the tubes of meat in hot lard. If any other processing medium, for which the rate of heat transfer is different, were used in the process bath, these corrections would not apply. The actual heating times, at four process temperatures, necessary to obtain processes having Fo values from one to 16 are listed (Table 1).

TABLE 1

Process Times to Obtain Fo Values of One to 16 for Ground Meat in 10 x 75 mm. Glass Tubes Processed in a Lard Bath

Fo value of process	Heating times at four bath temperatures			
	220°F.	230°F.	240°F.	250°F.
	min.	*min.*	*min.*	*min.*
1	48.35	14.97	5.77	3.24
2	94.77	27.89	9.37	4.34
3	141.19	40.81	12.96	5.34
4	187.61	53.73	16.56	6.34
5	234.03	66.65	20.15	7.34
6	280.45	79.57	23.74	8.34
7	326.87	92.49	27.34	9.34
8	373.29	105.41	30.93	10.34
9	419.71	118.33	34.53	11.34
10	466.13	131.25	38.12	12.34
11	512.55	144.17	41.71	13.34
12	558.97	157.09	45.31	14.34
13	605.39	170.01	48.90	15.34
14	651.81	182.93	52.50	16.34
15	698.23	195.85	56.09	17.34
16	744.65	208.77	59.68	18.34

Culturing Heat-Processed Tubes of Meat: The processed tubes were incubated directly, or their contents cultured into the brain medium described above. The tubes that were cultured after processing were opened by heating the upper portion of the tubes and touching the hot glass with a "culture-loop" of sodium hypochlorite solution. The meat was drawn from the opened tubes into culture medium with a bent culture

wire. Virtually no contamination was encountered when this procedure was followed.

A special device was arranged to allow one individual to perform the culturing of processed tubes of meat (Figs. 3 and 4). The device consists of a standard blast burner mounted on a board in such a position that the flame can be swung into position over the top of open-culture tubes placed upright in a cork borer held in place by fitting it on a rubber

FIG. 3. Apparatus used in connection with culturing processed tubes. Note position of flame over culture tube.

stopper fastened to the board (Fig. 3). When a tube of processed meat has been opened the flame can be directed upward by one simple movement of the hand and the meat readily drawn into the culture tube (Fig. 4).

DISCUSSION

Several methods for studying thermal resistance and growth of bacteria in canned foods have been reported by the American Can Company (1943). However, the method outlined here was chosen because it was believed that it presented certain advantages for the studies undertaken. These advantages are as follows: (1) it was possible to include wide variations in processes employed, wide variations in treatment of meats used, and replication of each study of variant combinations involved, without

the time and space required becoming prohibitive; (2) meat could be readily introduced, mechanically, into the glass tubes; (3) processed tubes of meat could be readily cultured into a medium favorable for growth of the test bacteria with very little danger of contamination; (4) unopened processed tubes of meat could be incubated conveniently (Fig. 5); and (5) progressive spoilage of the processed meat could be readily observed through the glass tubes incubated.

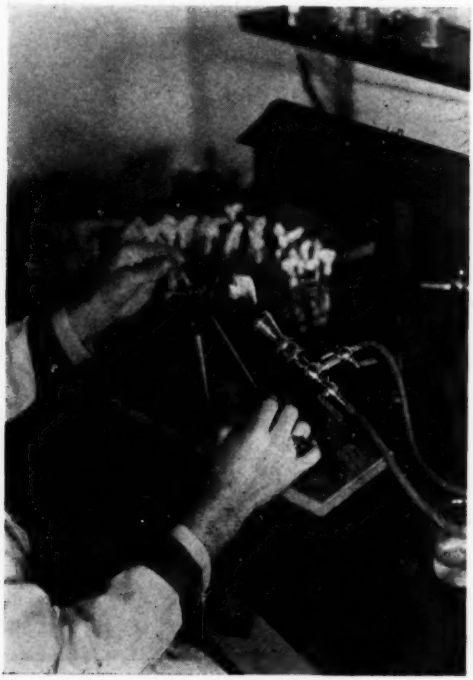

FIG. 4. Apparatus used in connection with culturing processed tubes. Note elevated position of flame and meat being drawn into culture tube.

The literature is replete with studies concerning the thermal resistance of bacteria. Practically every textbook, old or new, in general bacteriology discusses the comparative resistance of various bacteria. Factors which influence the resistance of individual species and even single strains of bacteria have been intensively studied. However, the literature is virtually void of information concerning the factors which influence thermal resistance of putrefactive anaerobes dispersed in meats. Numerous studies relating to thermal resistance of this group of bacteria in neutral phosphate solution have been reported, and information contained in many of such reports has been used quite widely by some investigators in arriving at processes to be used for canned meats.

The influence of various curing agents upon the thermal resistance and growth of bacteria in meat is not well understood. The influence of the number of bacterial spores upon thermal resistance of the spores in meat has not been studied. Again it would seem that the chief reason must be the lack of adequate methods for conducting such studies with meats. It should be possible by methods presented above, to collect valuable information concerning the influence of curing agents upon the keeping quality of heat-processed canned meats as well as the influence of these agents upon the heat processes required for sterilization.

FIG. 5. Unopened tubes of processed meat suspended from wires attached to incubator shelves.

Moreover, it should be possible by the same methods to determine, by routine examination of meats to be canned, the heat processes required to sterilize meats handled under conditions of routine packing-house operation. It is one of the most vital problems in the canning of meats to control the handling of meats, prior to canning, in such a manner that predetermined heat processes can be used with safety. Information to be obtained from flow-sheet analyses of heat processes required to sterilize canned meats should prove highly valuable. Flow-sheet analyses of F values required for meats in various stages of product manufacture should facilitate locating sources of contamination with heat-resistant bacteria. Also, by similar methods, it should be possible to determine the influence of added ingredients upon the sterilizing values required for finished meat products.

By virtue of evaluating the experimental processes in terms of Fo, the procedure of applying results to practice is greatly simplified; for example, if by flow-sheet analysis it has been found that over a long period of time a given Fo is required to sterilize a given meat product, the evaluation of a process for the given product in terms of time and retort temperature is,

if the heat-penetration curve of the product is known, a simple mathematical or graphical procedure.

The methods outlined in this paper are being used in this laboratory in studies designed to collect some of the information vital to accurate evaluation and intelligent application of heat processes for canned meats. They are offered here with the hope that they may prove of value to others in studying various bacteriological phases of the heat-processing problem.

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EFFECT OF SPICES AND FLAVORING MATERIALS ON GROWTH OF YEASTS

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Spices have played an interesting role in the changing history of the world and in the lives of most individuals. It is believed that their value as condiments was discovered in prehistoric times, and likewise at some early period preservative properties were noticed. The Ebers Papyrus, the earliest known medical record, attests to use of spices among the Egyptians about 1550 B.C., and subsequent writings detail information as to their employment in food preservation and embalmery. In keeping with wider applications of cold as a preservative, importance of spices as preservatives of foods declined almost to the vanishing point, although a little interest has been revived lately in practical food technology. Blum and Fabian (1943) advocated use of spices and their essential oils in preventing microbial scum formation on such commercial products as pickles, vinegars, and bottled wines.

Leeuwenhoek found millions of microorganisms in an infusion of whole peppers. The significance of this discovery has been overlooked, and through intervening centuries all investigation into efficacy of spice preservation has begun with the assumption that spices, generally, are inimical to microbial life. James (1931) was apparently the first investigator to question antiseptic ability of spices. He grew *Escherichia coli* in nutrient broths containing 0.62 per cent ground mustard and 0.5 per cent ground cinnamon and cloves. His results cast serious doubt on the practicability of favorably accepted food preservatives to prevent growth of *Escherichia coli*. Jensen, Wood, and Jansen (1934), investigating an outbreak of swelling in a canned meat product, counted 5,000,000 aerogenic bacilli per gram in the meat, apparently contributed by coriander and white pepper. Hall (1938); James (1938); Fabian, Krehl, and Little (1939); and Yesair and Williams (1942) recorded large numbers of microorganisms per gram on whole and ground spices. Such reports would seem to belittle the reputation of spices as preservatives of foods and other products.

In experiments designed to prove their inhibitory properties, spices have fared better. Chamberland (1887) demonstrated the lethal effect of cinnamon oil for anthrax spores and was able to produce sublethal stasis with other common spice oils. Martindale (1910) and Penfold and Grant (1922-26) established phenol coefficients for a number of oils but were not able to agree on their values. Grove (1918) preserved tomato sauce successfully with aqueous and alcoholic extracts of ground cinnamon and cloves; and Prasad and Joshi (1929), in India, recommended a practical method of preserving native fruits using infusion of ground cloves in combination with salt. Cochran and Perkins (1914), Hoffman and Evans (1911), and Corran and Edgar (1933) observed germicidal action of spices and essential oils in food preservation and in *in vitro* experiments.

In most experimental work on inhibitory properties of spices and essential oils, bacteria have been employed as test organisms. Reactions of yeasts to environmental conditions are not always identical with those of the bacteria, and as conditions under which spices and oils are effective inhibitors of bacterial growth have been fairly well established, it seemed of interest to determine effects of spices on yeasts. In two of the experiments reported here yeasts were grown on spiced agar with the "divided plate" technique of Frost. This method became popular following publication by Churchman (1912) on selective action of dyes as disinfectants, and the method has generally been credited to him. However, Frost (Frost and Bachmann, 1916) pointed out that he employed the technique eight years earlier than Churchman (Frost, 1904) and believed that Churchman was unfamiliar with the earlier paper. On the basis of priority it seems justifiable to award credit to Frost and to adopt his name for the "double plate" described by him in 1904.

GROWTH OF YEASTS IN SPICE INFUSIONS

Commercial grades of whole and ground spices were prepared in 10-gram quantities and infused in 10 milliliters of distilled water. These 10-per cent infusions were steamed in the autoclave in order to sterilize them and to extract essential oils, as recommended by Fabian, Krehl, and Little (1939). Dilutions of 1-50 and 1-100 were made in dextrose broth of pH 6.8 in fermentation tubes and inoculated with drop quantities of 36-hour cultures of yeasts. In all, 11 spices were tested with eight yeasts representing six common genera. The culture tubes were incubated seven days at a room temperature of 25°C.(77°F.) and inhibition or presence of growth, as indicated by clouding or fermentation of broth, was recorded.

Cinnamon, cloves, allspice, bay leaves, and mixed spices were inhibitive in 1-50 dilution; cinnamon was most effective, preventing six of the eight yeasts from growing (Table 1). Clove infusion inhibited five yeasts, allspice two, and bay leaves and mixed spices one each. In 1-100 dilution cinnamon and cloves alone were inhibitory, suppressing growth of six and five yeasts, respectively. *Oidium lactis* and *Saccharomyces hansenii*, the two species recorded resistant to cinnamon, were somewhat retarded by this spice in 1-50 concentration but were unaffected by the higher dilution. In order to determine whether or not the yeasts were killed, subcultures of tubes showing no growth were made into fresh broth in such a manner that spice infusion carried over was too highly diluted to produce static effect. Cinnamon and cloves were apparently lethal in action; no subsequent growth was observed. After three days' incubation *Oidium lactis*, subcultured from infusion by bay leaves, was able to produce clouding in the medium indicating some measure of survival. Microscopic examination proved it to be pure and identical in morphology with the original inoculum.

On the basis of this experiment all spices except cinnamon and cloves could be declared to have no, or at best very little, inhibitory action as infusions. In order to further study activity of these effective inhibitors, infusions were added to dextrose broth in quantities to render final dilutions of 1-200, 1-400, 1-600, 1-800, and 1-1,000. These preparations were

inoculated with the eight yeasts as in the previous experiment and incubated seven days at room temperature. In 1-600 dilution, beyond which cloves were not effective, cinnamon inhibited four yeasts, three in 1-800 dilution and one in 1-1,000 dilution. Clove infusion prevented growth of four yeasts in 1-200 dilution and in 1-400. Two yeasts only were inhibited in 1-600 dilution; this was the limit of inhibitory action. Subcultures from tubes showing no growth remained sterile, indicating that no cells survived in the original cultures despite low concentrations of spice infusions.

TABLE 1

Growth of Yeasts in 1-50 and 1-100 Spice Infusions

Spice	*Zygosaccharomyces priorianus*		*Oidium lactis*		*Torula sphaerica*		*Saccharomyces cerevisiae*		*Mycoderma vini*		*Saccharomyces hansenii*		*Schizomycetes mellacei*		*Zygosaccharomyces pastori*	
	A[1]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Cinnamon	—	—	—	—	—	±	±	+	—	—	—	—	—	—	—	—
Clove	—	—	+	+	+	+	—	+	—	—	+	+	—	—	+	+
Allspice	±	±	—	+	+	+	—	+	+	+	±	+	—	+	—	±
Bay leaves	—	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+
Mixed spices	±	+	±	+	+	+	+	+	—	+	+	+	+	+	±	+
Mustard	±	+	±	+	±	+	±	+	±	+	+	+	±	+	±	+
Red pepper	±	+	±	+	±	+	±	+	±	+	+	+	±	+	+	+
Black pepper	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ginger	±	+	+	+	+	+	±	+	±	+	±	+	+	+	+	+
Nutmeg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Paprika	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(A)=1-50 dilution; (B)=1-100 dilution; (+)=growth; (—)=no growth; (±)=slight growth.

GROWTH OF YEASTS ON SPICE-AGAR PLATES

Frost double plates were prepared by fitting petri dishes with cardboard partitions and sterilizing them in the hot-air oven. Dextrose agar was poured on one side of the cardboard partitions; when hard, partitions were loosened with a flamed spatula and removed with sterilized forceps. The plates were incubated 24 hours to test sterility, and agars containing various percentages of ground spices were poured in the remaining parts of the dishes. Care was taken so that the two sides of the plates composed a smooth level surface. Plates were inoculated by making a single streak of a 36-hour broth culture of a yeast from the outer edge of the control surface to the center line, recharging the loop, and inoculating the spiced agar as a projection of the original line. Thereby an effort was made to have roughly the same degree of inoculum on both sides of the plate. Several species were streaked on one plate and plates were incubated five days at room temperature.

Cinnamon, cloves, and allspice in 0.1 per cent concentration inhibited yeast growth in a marked degree. Cinnamon seemed to be most effective and cloves were little better than allspice. The other spices supported luxuriant growths. In one-per cent concentration mixed spices were negative in effect. In plates containing inhibitive spices the retarding principle, presumably the essential oils, diffused from the spiced into the unspiced

agar. This was most noticeable in case of cinnamon and cloves; the agar was darkened and growth of inoculum was stopped one or two inches from the line of demarcation. In all instances of marked inhibition the yeasts formed spearheads of resistant growth into the area of inhibition. Cinnamon and cloves were so inhibitory in this series that growth of yeasts was stopped far back from the spiced agar and was confined to a narrow area at the peripheries of the plates. This concentration did not affect normal luxuriant growth of yeasts on other spices with the exception of mixed spices which showed increased inhibitory effect. Higher concentrations of 10 and 20 per cent did not alter results already observed; cinnamon, cloves, allspice, and mixed spices were inhibitory; other spices supported luxuriant growths as in lower concentrations.

GROWTH OF YEASTS ON SPICE-OIL PLATES

An experiment paralleling that with ground spices was set up incorporating spice essential oils in Frost double plates. Plates were prepared by delivering an appropriate quantity of oil in the bottom of a sterile tube and adding 10 ml. of hot dextrose agar. This was shaken vigorously and quickly poured into a partitioned petri dish previously poured with unspiced dextrose agar. An effort was made to separate oils into as fine droplets as possible so that a smooth emulsion would be effected. Inoculum was prepared and streaked as in the previous experiment and plates incubated five days at room temperature. Oils of cinnamon, mustard, cloves, allspice, bay, wintergreen, and peppermint in 0.1 per cent concentration retarded and, in most instances, completely inhibited growth of inoculum. On a basis of total number of species completely inhibited, bay was least effective of the inhibitory oils. Oils of almond, ginger, nutmeg, the peppers, lemon, onion, and an alcoholic extract of vanilla were without inhibitory effect. In one-per cent concentration almond alone of the spice oils not showing inhibition in 0.1 per cent was active. In amounts of five per cent most oils ineffective in lower concentrations became active; almond increased in antiseptic effect as did lemon. In 10-per cent concentration all oils except the peppers effected more-or-less retardation on spiced agars with, in all instances, inhibitory effect extending to control surfaces. In this series the antiseptic effect was due as well to saturation of the atmosphere of the plates by spice oils as to diffusion through the agar medium. Agars on control sides were darkened by the oils, and the plates gave off strong, spicy odors.

As these plates were examined the ones showing positive inhibition were set aside for three weeks to observe effects of drying. At the end of this period oils in 0.1-per cent concentration had evaporated from the medium and allowed subsequent growth of yeasts and mold contaminants. From this it may be inferred that oils in this low concentration were bacteriostatic in effect rather than germicidal. The yeast cells were inactivated but not killed by presence of spice oils and resumed development as oils were lost through evaporation. Over the same period plates impregnated with mustard, cinnamon, cloves, anise, allspice, and wintergreen oils in one-per cent concentration developed no new growths on spiced agars and no new growth on controls, indicating that yeast cells originally streaked

on the spiced agars had been killed. Peppermint, bay, and almond oils were apparently not lethal in this concentration, new growth appearing as oils were lost. Almond and onion oils in five-per cent concentration permitted no new growth in contrast to lower dilutions; results of the 10-per cent series followed the same trend; no additional oils demonstrated lethal effects. Results of this experiment indicated that oils of mustard, cinnamon, and cloves were definitely germicidal in concentrations above one per cent; anise, allspice, onion, and wintergreen oils were probably germicidal above five per cent; peppermint, bay, and ginger oils were questionable in effect; and the peppers were innocuous.

EFFECT OF SPICE OILS ON YEASTS AS DETERMINED BY CUP-PLATE METHOD

For this work a modification of the cup-plate method of Himebaugh, as described by Reddish (1929), was employed in order that its several unique features could be utilized in studying inhibitory action of spice oils. In the same plate can be created conditions which can be interpreted as being either germicidal or bacteriostatic; and by subculture from the clear area surrounding cup and test substance, one or the other can be substantiated. Pour plates of dextrose agar heavily seeded with yeasts were made, and plugs were cut from the centers with a sterile cork borer. Twenty-five hundredths of a milliliter of spice oils was added to the cups, and plates were incubated five days at room temperature. After incubation, zones of inhibition were measured in centimeters and subcultures made from clear areas into dextrose broth. Ten trials were made with each of the 16 oils and a mean calculated of the radii of clear areas surrounding the cup and oil. This was expressed as the penetration value of the oil. In case of mustard, which completely suppressed the yeasts, an artificial mean was established by measuring the radius of a plate; this figure was 4.5 cm. and was used as the penetration value of the oil. Oils of cinnamon, allspice, bay, and wintergreen had values above one. Other oils ranged below one down to black pepper which with a value of zero was incapable of producing a clear zone of inhibition about the cup. Likewise, a value was determined which represented the quotient of the number of negative subcultures divided by the number of positive subcultures from zones of inhibition in a single spice-oil series. This figure was called the inhibition value. The oils of cinnamon, mustard, cloves, and bay had values of 10, indicating that these oils killed all yeasts subjected to test. Black pepper, white pepper, and ginger had values of zero, having either no clear areas of inhibition or yielding positive subcultures in all instances.

It should be pointed out that while this method is a recognized one for testing efficacy of antiseptics, its most outstanding feature is that it tests ability of antiseptics to penetrate through agar. There was apparently little or no correlation between depth of penetration of oil and germicidal activity. For example, almond oil with a penetration value of 40 cm. and oil of bay with a value of 1.09 cm. both gave inhibition values of 10, the highest possible, and rank in the same category with mustard, cinnamon, cloves, and allspice. The extent to which saturation of the atmosphere by vapor of the oils aids in establishing the zone of inhibition could not be

determined in an experiment of this sort, but indications from other experiments from work of Lebduska and Pidra (1940) are that saturation of the atmosphere above the medium constitutes as great a check on growth of inoculum as saturation of the medium itself.

Under conditions of this experiment it was indicated that oils of mustard, cinnamon, cloves, allspice, almond, and bay were germicidal to yeast cells. Oils of anise, peppermint, wintergreen, lemon, and onion were in some instances germicidal but may best be classed as bacteriostatic. Oils of ginger, black and white peppers, and an alcoholic extract of vanilla were neither germicidal nor bacteriostatic.

DISCUSSION

The parallel experiments testing inhibitory action of ground spices and spice essential oils would not seem to be well correlated. It may best be assumed that an active antiseptic principle is contained in essential oils, and discrepancies between the action of ground spice and spice oil can be explained by percentage by weight of active principle in ground spice and oil. In commercial processes 1.25 kilograms of essential oil can be extracted from 100 kilograms of cinnamon bark, 19 kilograms of clove oil from a like amount of whole cloves, and only 0.75 kilogram of oil from whole mustard seeds. It follows from this that the antiseptic principle is concentrated manyfold more in a given amount of oil than in the same weight of ground or whole spice; therefore, parallel experiments employing in the one whole or ground spice and in the other spice essential oils will produce results which although comparable need not be identical. James (1931) found one sample of old ground mustard to be without antiseptic effect, which is indication that ground spices on standing may in large measure lose their volatile antiseptic principles.

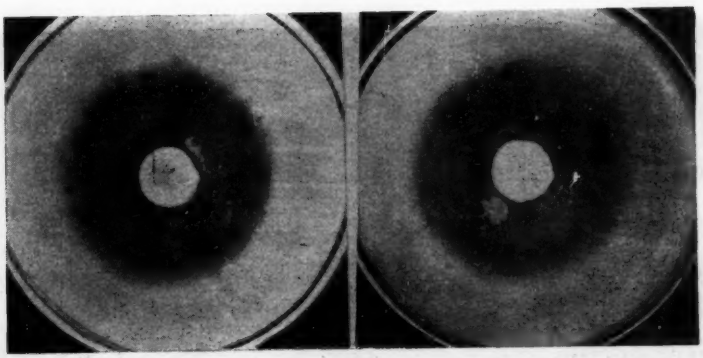
It would be of interest to discover the disinfectant mechanism of the essential oils. Brannt (1896) hypothesized that germicidal action of essential oils is due to presence of terpenes of the formula $C_{10}H_{16}$. These terpenes, widely distributed in essential oils, oxidize under influence of water to liberate hydrogen peroxide which serves as the actual disinfecting agent. According to this view, ginger and the peppers, which have high terpene constituency, should rank among the stronger germicides. However, in the experiments reported here, the peppers and ginger were almost negative in effect against test yeasts. Myers (1926) pointed out that chemical classification of essential oils gave no clue to fungicidal activity, and that view is applicable here. Examining the hypothesis that oxidation of terpenes liberates H_2O_2, it is found that ginger, peppers, lemon, peppermint, and nutmeg contain terpenes of the formula $C_{10}H_{16}$ and are not antiseptic in any appreciable degree. Among the most efficient germicides are cinnamon, cloves, and allspice which do not contain terpenes $C_{10}H_{16}$, but contain the ether, eugenol, $C_6H_3OH\ (OCH_3)\ C_3H_5 = C_{10}\ H_{12}O_2$, which is in itself highly germicidal and may be the active principle of the oils. Oils of mustard, onion, and garlic contain sulfur and are as a group germicidal.

Photographs were prepared by a method described by Buchholz and Lewis (1930); a petri dish was used as the photographic negative; sensitized paper was exposed in the dark room and developed as a positive

Control, no spice | 10 per cent allspice | Control, no spice | 20 per cent allspice

Control, no spice | One per cent cloves | Control, no spice | Five per cent cloves

Control, no spice | One per cent cinnamon | Control, no spice | Five per cent cinnamon



Clove oil | Allspice oil

Zones of inhibition with spice oils

print. Petri dishes were placed over Eastman Apex S-2 paper, exposed two seconds to rays of a 100-watt bulb at a distance of two feet and developed in Eastman D-72 developer, using an acid fixer. Prints were dried on a ferrotyping plate and were comparable to plates made with lens and camera.

SUMMARY AND CONCLUSIONS

Water infusions of ground cinnamon, cloves, and allspice prevented growth of yeasts in broth cultures in 1-50 concentration. Infusions of bay leaves, mustard, paprika, ginger, black and red peppers, and nutmeg did not prevent growth of yeasts. Cinnamon completely inhibited one species of yeast in 1-1,000 dilution.

One-tenth per cent by weight of ground cinnamon, cloves, and allspice in dextrose agar markedly inhibited but did not prevent growth of test yeasts. Nutmeg, bay leaves, paprika, ginger, and peppers prevented growth in concentrations as high as 10 per cent.

Oils of cinnamon, cloves, and mustard were germicidal in agar plates in concentrations above one per cent. Oils of anise, allspice, wintergreen, and onion were germicidal in concentrations above five per cent; bay and ginger oils were in most instances bacteriostatic in all concentrations. Oils of black and white peppers appeared to contain growth stimulants for the yeasts.

Oils of cinnamon, cloves, allspice, almond, and bay were germicidal when tested by the cup-plate technique. Yeasts demonstrated a strong species specificity to disinfectant action. *Monilia candida* and *Mycoderma lactis* survived contact with anise and wintergreen oils which were lethal to other organisms.

Spice oils, because of greater concentrations of active principle, were more direct in disinfectant action than were whole or ground spices. Yeasts demonstrated adaptative responses to spice disinfectants, as evidenced by spearhead growth into zones of inhibition on spiced agars. Spices are effective inhibitors of yeast growth under some conditions, but yeasts can readily adapt themselves to low concentrations of spices and oils and in some instances appear to be stimulated by them.

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BACTERIOLOGICAL STUDIES RELATING TO THERMAL PROCESSING OF CANNED MEATS

II. INFLUENCE OF MEAT-CURING AGENTS UPON THERMAL RESISTANCE OF SPORES OF A PUTREFACTIVE ANAEROBIC BACTERIUM IN MEAT

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There is very little information available relative to the influence of sugar (sucrose), sodium chloride, sodium nitrate, and sodium nitrite upon the thermal resistance of bacteria dispersed in meat. Various combinations of some or all of these agents have long been employed in curing meats because of the recognized influence of permissible concentrations of some agents upon the quality of meats, especially upon keeping quality. With reference to heat-processed canned meats there seems to be some question whether the influence on keeping quality is due solely to inhibitory effects of the agents upon bacteria surviving heat processes, or whether the influence is due in part to lethal action of the agents upon bacteria during the heat processes.

Yesair and Cameron (1942) found in their studies with spores of *Clostridium botulinum* dispersed in meat that thermal resistance values, determined by culturing processed meat into a favorable medium for the growth of the organism, were approximately equal in cured and uncured meat. The curing agents used were as follows: NaCl — 3.5 per cent, $NaNO_3$—0.172 per cent, $NaNO_2$—0.0156 per cent, and sugar (sucrose) two per cent. The data presented indicate that the influence of curing agents upon keeping quality of meats may be solely due to inhibitory effects of the agents. However, Jensen (1942) states, "Perhaps in the presence of nitrate and nitrite, and salt as well, the spores of *Clostridium botulinum* as well as its close 'relative,' *Clostridium sporogenes,* are killed at much lower temperatures than called for in standard canning practice."

Methods for studying the influence of various factors upon thermal resistance and growth of bacteria in canned meats were presented by Stumbo, Gross, and Vinton (1944). It is the purpose of this paper to present results of studies concerning the thermal resistance of spores of a putrefactive anaerobe dispersed in meat.

EXPERIMENTAL PROCEDURE

The general procedures presented by Stumbo, Gross, and Vinton (1944) were followed throughout the investigation. Fresh trimmings (blade meat, butts, picnics) were ground through a 1/8-inch plate. The ground meat was then mixed thoroughly in a mechanical mixer. Various batches of meat and various portions of each batch of meat were treated differently

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with curing agents and inoculum. The curing agents employed and the amounts of each used were as follows: sodium chloride, 3½ pounds per 100 pounds of meat (designated in tables as salt—3½); sodium chloride, 1½ pounds per 100 pounds of meat (designated in tables as salt—1½); sugar, 1 pound per 100 pounds of meat; sodium nitrate, 2¾ ounces per 100 pounds of meat; and sodium nitrite, ¼ ounce per 100 pounds of meat. The inoculum employed consisted of a uniform suspension of spores of the putrefactive anaerobe S_2 described in the above-mentioned paper concerning methods. Curing agents and inoculum were added to quantities of the ground meat and thoroughly mixed (15 minutes) with the meat under vacuum in a mechanical mixer. All tubes of meat prepared from the variously treated batches of meat were heat processed 24 hours after the mixing operation. During this 24-hour period the tubes of meat were kept at approximately 3.3°C.(38°F.), a temperature commonly recommended for curing meats to be canned.

After the tubes of meat were processed, the meat in each tube was cultured into a tube of brain medium. The culture tubes were then incubated at 28°C.(82.4°F.) for 11 days. During this time periodic examinations were made for evidence of growth of the test organism. At the end of this incubation period all culture tubes in which growth had not occurred were transferred to a 37°C.(98.6°F.) incubator and incubation was continued there for four days. Growth of the test organism, if occurring during this 15-day incubation period, usually started within four days. Growth in only one or two tubes per thousand started after the seventh day of incubation.

The data shown (Tables 1 to 7) were obtained from culturing tubes of meat subsequent to heat processing them as designated in the left-hand columns of the tables. Each plus sign indicates positive growth of the test organism in one culture tube containing the contents of one processed tube of meat; each minus sign indicates the opposite.

It is clearly evident from the data (Tables 1 to 7) that none of the curing agents employed appreciably influenced thermal resistance of the spores of putrefactive anaerobe S_2 when the spores were present in concentrations of 5, 10, 100, 500, 1,000, and 10,000 per gram of meat. The data would seem to indicate slightly higher resistance for spores in meat containing sodium chloride and sodium nitrate. However, the evidence is not sufficient to warrant any definite conclusion. These results tend to confirm the studies upon spores of *Clostridium botulinum* reported by Yesair and Cameron (1942). The results of both studies indicate that curing agents in concentrations permissible to use in curing meat cannot be depended upon to aid the heat of processes in sterilizing canned meats.

The influence of the number of spores of the anaerobe per gram of meat upon the process required to sterilize the meat is evident upon comparison of the data obtained for different spore concentrations.

It should be pointed out that at the time the tubes of meat were cultured for thermal resistance studies reported above a duplicate lot of tubes was placed under incubation at 28°C. These tubes were placed in the incubator December 18, 1943, and periodic examinations for evidence of spoilage of the meat are being made and will continue to be made for a

TABLE 1

Growth in Cultures From Tubes of Processed Uninoculated Meat (Control)

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_3$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_2$, $NaNO_3$	Sugar, salt–1½, $NaNO_2$, $NaNO_3$
min.											
5.77	1	— —	— —	+ +	— —	— —	— —	+ —	+ +	— —	— —
9.37	2	— —	— —	— —	— —	— —	— —	+ —	— —	— —	— —
12.96	3	— —	— —	— —	+ —	— —	— —	— —	— —	— —	— —
16.56	4	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
20.15	5	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
23.74	6	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
27.34	7	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
30.93	8	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
34.53	9	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
38.12	10	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
41.71	11	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
45.31	12	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
48.90	13	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
52.50	14	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
56.09	15	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
59.68	16	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —

TABLE 2

Growth in Cultures From Tubes of Meat Inoculated With Five Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_3$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_2$, $NaNO_3$	Sugar, salt–1½, $NaNO_2$, $NaNO_3$
min.											
5.77	1	++	++	++	++	++	++	++	++	++	++
9.37	2	++	++	++	++	++	++	++	++	++	++
12.96	3	+—	++	++	++	++	+—	++	++	++	+—
16.56	4	+—	——	++	++	++	+—	++	++	+—	+—
20.15	5	+—	——	+—	+—	++	——	——	+—	++	+—
23.74	6	——	——	——	+—	+—	——	——	+—	——	——
27.34	7	——	——	——	——	+—	——	——	——	——	——
30.93	8	——	——	——	——	——	——	——	——	——	——
34.53	9	——	——	——	——	——	——	——	——	——	——
38.12	10	——	——	——	——	——	——	——	——	——	——
41.71	11	——	——	——	——	——	——	——	——	——	——
45.31	12	——	——	——	——	——	——	——	——	——	——
48.90	13	——	——	——	——	——	——	——	——	——	——
52.50	14	——	——	——	——	——	——	——	——	——	——
56.09	15	——	——	——	——	——	——	——	——	——	——
59.68	16	——	——	——	——	——	——	——	——	——	——

TABLE 3

Growth in Cultures From Tubes of Meat Inoculated With 10 Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_3$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_2$, $NaNO_3$	Sugar, salt–1½, $NaNO_2$, $NaNO_3$
min.											
5.77	1	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
9.37	2	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
12.96	3	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
16.56	4	+ —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ —
20.15	5	— —	+ +	+ —	+ +	+ +	+ +	+ +	+ —	+ —	— —
23.74	6	— —	— —	+ —	+ —	+ +	+ +	+ —	+ +	— —	— —
27.34	7	— —	— —	+ —	+ +	+ +	— —	+ —	+ —	+ —	— —
30.93	8	— —	— —	— —	— —	+ +	— —	— —	— —	— —	— —
34.53	9	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
38.12	10	— —	— —	+ —	— —	— —	— —	— —	— —	— —	— —
41.71	11	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
45.31	12	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
48.90	13	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
52.50	14	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
56.09	15	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
59.68	16	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —

TABLE 4

Growth in Cultures From Tubes of Meat Inoculated With 100 Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_3$	Sugar, $NaNO_3$, $NaNO_3$	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_3$, $NaNO_2$	Sugar, salt–1½, $NaNO_3$, $NaNO_2$
min.											
5.77	1	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
9.37	2	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
12.96	3	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
16.56	4	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
20.15	5	+ —	+ —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ —
23.74	6	— —	+ —	+ +	+ +	+ +	+ +	+ +	+ +	+ —	— —
27.34	7	— —	— —	+ +	+ +	+ +	+ —	+ —	+ —	— —	— —
30.93	8	— —	— —	+ +	+ —	+ —	— —	+ —	— —	— —	— —
34.53	9	— —	— —	— —	+ —	+ —	— —	— —	— —	— —	— —
38.12	10	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
41.71	11	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
45.31	12	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
48.90	13	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
52.50	14	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
56.09	15	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
59.68	16	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —

TABLE 5

Growth in Cultures From Tubes of Meat Inoculated With 500 Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3 ½	Sugar, $NaNO_2$	Sugar, salt–3 ½, $NaNO_3$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_2$	Sugar, salt–3 ½, $NaNO_2$	Sugar, salt–3 ½, $NaNO_2$, $NaNO_3$	Sugar, salt–1 ½, $NaNO_3$, $NaNO_2$
min.											
5.77	1	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
9.37	2	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
12.96	3	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
16.56	4	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
20.15	5	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
23.74	6	+ —	+ +	+ +	+ +	+ +	+ —	+ +	+ +	+ +	+ +
27.34	7	+ —	+ +	+ +	+ +	+ +	+ —	+ +	+ +	— —	+ +
30.93	8	— —	— —	— —	+ —	+ +	— —	+ —	— —	— —	+ —
34.53	9	— —	— —	— —	+ —	+ +	— —	— —	— —	— —	— —
38.12	10	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
41.71	11	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
45.31	12	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
48.90	13	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
52.50	14	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
56.09	15	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
59.68	16	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —

TABLE 6

Growth in Cultures From Tubes of Meat Inoculated With 1,000 Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_2$, $NaNO_3$	Sugar, salt–1½, $NaNO_2$, $NaNO_3$
min.											
5.77	1	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
9.37	2	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
12.96	3	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
16.56	4	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
20.15	5	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
23.74	6	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ —	+ —
27.34	7	+ —	+ +	+ +	+ +	+ +	+ —	+ +	+ —	+ +	+ —
30.93	8	— —	+ —	+ —	+ +	+ +	— —	+ —	+ +	— —	— —
34.53	9	— —	— —	— —	+ +	— —	— —	+ +	+ —	— —	— —
38.12	10	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
41.71	11	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
45.31	12	— —	— —	— —	— —	— —	— —	— —	+ —	— —	— —
48.90	13	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
52.50	14	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
56.09	15	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
59.68	16	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —

TABLE 7

Growth in Cultures From Tubes of Meat Inoculated With 10,000 Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_2$, $NaNO_3$	Sugar, salt–1½, $NaNO_2$, $NaNO_3$
min.											
5.77	1	++	++	++	++	++	++	++	++	++	++
9.37	2	++	++	++	++	++	++	++	++	++	++
12.96	3	++	++	++	++	++	++	++	++	++	++
16.56	4	++	++	++	++	++	++	++	++	++	++
20.15	5	++	++	++	++	++	++	++	++	++	++
23.74	6	++	++	++	++	++	++	++	++	++	++
27.34	7	++	++	++	++	++	++	++	+—	++	++
30.93	8	++	++	++	++	++	++	++	——	+—	++
34.53	9	+—	+—	++	++	++	+—	+—	——	——	++
38.12	10	——	——	+—	++	++	——	——	——	——	+—
41.71	11	——	——	——	——	++	——	——	——	——	——
45.31	12	——	——	+—	——	+—	——	——	——	——	——
48.90	13	——	——	——	——	++	——	——	——	——	——
52.50	14	——	——	——	——	——	——	——	——	——	——
56.09	15	——	——	——	——	——	——	——	——	——	——
59.68	16	——	——	——	——	——	——	——	——	——	——

period of at least one year. The results of this study should reveal the influence of the curing agents upon the growth of the organism in meat. These results will be published when available.

SUMMARY

The curing agents commonly employed for curing meats to be canned did not influence the heat processes necessary to sterilize meat inoculated with known concentrations of spores of a putrefactive anaerobe similar in many respects to *Clostridium sporogenes* and *Clostridium botulinum*. The higher the concentration of spores in the meat, the more severe were the heat processes necessary to sterilize it.

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BACTERIOLOGICAL STUDIES RELATING TO THERMAL PROCESSING OF CANNED MEATS

III. INFLUENCE OF MEAT-CURING AGENTS UPON GROWTH OF A PUTREFACTIVE ANAEROBIC BACTERIUM IN HEAT-PROCESSED MEAT

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Available literature concerning the influence of meat-curing agents upon growth of putrefactive anaerobes is in many respects controversial. In most reported studies the inhibitory effects of the agents have been determined by incorporating varying concentrations of the agents in prepared media, inoculating the media with spores of the test bacteria, and observing the effects of the agents upon germination of spores and growth of the bacteria—Tanner and Evans (1933, 1934), Jensen (1942), and Yesair and Cameron (1942). Tanner (1933) pointed out, with reference to the growth of *Clostridium botulinum* in different menstrua, that the inhibiting concentration of sodium chloride seems to be influenced by the medium in which the experiments are carried out. Though no information is available on the point, it may be that the same is true concerning the influence of curing agents other than salt. If this is found to be true it may serve to explain some of the apparently contradictory opinions concerning the efficacy of certain meat-curing agents in inhibiting growth of putrefactive anaerobic bacteria in meat.

The studies reported here have been conducted under conditions simulating as nearly as possible those conditions under which comminuted canned meat is manufactured. Methods for studying the influence of various factors upon thermal resistance and growth of bacteria in canned meats were presented by Stumbo, Gross, and Vinton (1944a). In a second paper of this series the same authors (1944b) reported the results of a study concerning the influence of meat-curing agents upon thermal resistance of spores of a putrefactive anaerobic bacterium dispersed in meat. It is the purpose of this paper to present the results of a study concerning the influence of meat-curing agents upon the growth of this same organism (putrefactive anaerobe S_2) in heat-processed meat.

EXPERIMENTAL PROCEDURE

The tubes of meat upon which results are reported here were duplicates of those cultured in the thermal resistance studies reported by Stumbo, Gross, and Vinton (1944b). Therefore, the data presented in the second paper will serve to indicate where growth should have occurred in the incubated tubes of meat, the results from which are recorded below, should no inhibition of growth occur. Further, the methods outlined by Stumbo,

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Gross, and Vinton (1944a, b) were employed in the preparation and processing of all tubes of meat and need not be repeated here.

The processed tubes of meat were placed in a 28°C.(82.4°F.) incubator December 18, 1943. All tubes were examined periodically for evidence of spoilage as indicated by gas production and color changes in the meat and by digestion of the meat. Spoilage observed in each tube is represented (Tables 1 to 7) by a letter corresponding to the time the observation was made. The times of observation and the letter indicating spoilage at these times are as follows:

Time of observation after beginning of incubation	Letter indicating observed spoilage
2 days	a
6 days	b
11 days	c
18 days	d
32 days	e
40 days	f
54 days	g
77 days	h
111 days	i
249 days	j
365 days	k

SUMMARY

Conclusions regarding the inhibitory effect of curing agents were based on periodic visual observations of the incubated tubes over a period of one year. The data would seem to support the following statements: (1) Sugar (1 pound per 100 pounds of meat) alone was ineffective in preventing spoilage of the meat; (2) sodium nitrate (2¾ ounces per 100 pounds of meat) alone was ineffective in preventing spoilage; (3) sodium nitrite (¼ ounce per 100 pounds of meat) alone was ineffective in preventing spoilage but did appreciably delay germination; (4) salt in a concentration of 3½ pounds per 100 pounds of meat seemed to inhibit spoilage of all but a few tubes of meat for at least one year, while in a concentration of 1½ pounds per 100 pounds of meat it has not inhibited spoilage to the same degree; (5) salt and sodium nitrite together; salt and sodium nitrate together, or salt, sodium nitrite, and sodium nitrate together have thus far proved very little if any more effective in preventing spoilage than salt alone; and (6) the data show that over a period of one year sodium nitrate, either alone or in combination with other curing ingredients, has not appreciably influenced spoilage and, therefore, growth of putrefactive anaerobe S_2 in the meat.

TABLE 1

Progressive Spoilage in Incubated Tubes of Processed Uninoculated Meat (Control)

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3 ½	Sugar, $NaNO_2$	Sugar, salt–3 ½, $NaNO_2$	Sugar, $NaNO_3$, $NaNO_2$	Sugar, $NaNO_2$	Sugar, salt–3 ½, $NaNO_2$	Sugar, salt–3 ½, $NaNO_3$, $NaNO_2$	Sugar, salt–1 ½, $NaNO_3$, $NaNO_2$
min.											
5.77	1				ac						
9.37	2							ij			
12.96	3							i			i
16.56	4				c						
20.15	5										
23.74	6										
27.34	7										
30.93	8										
34.53	9										
38.12	10										
41.71	11										
45.31	12										
48.90	13										
52.50	14										
56.09	15										
59.68	16										

TABLE 2

Progressive Spoilage in Incubated Tubes of Meat Inoculated With Five Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_3$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_3$, $NaNO_2$	Sugar, salt–1½ $NaNO_3$, $NaNO_2$
min.											
5.77	1	aa	aa	hi	aa		eh	bi	hh		fh
9.37	2	bb	aa	h	ab	ee	bh	hi			i
12.96	3	bb	ab		bb	i	ii	ii			
16.56	4	bf	bb	i	ab		ii	h			i
20.15	5		be		ee		i	g			
23.74	6	h	eg		eg		i				
27.34	7		hh		h						
30.93	8										
34.53	9				e						
38.12	10										
41.71	11										
45.31	12										
48.90	13										
52.50	14										
56.09	15										
59.68	16										

TABLE 3

Progressive Spoilage in Incubated Tubes of Meat Inoculated With 10 Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3 ½	Sugar, $NaNO_3$	Sugar, salt–3 ½, $NaNO_3$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_2$	Sugar, salt–3 ½, $NaNO_2$	Sugar, salt–3 ½, $NaNO_2$, $NaNO_3$	Sugar, salt–1 ½, $NaNO_2$, $NaNO_3$
min.											
5.77	1	aa	aa	hi	aa	eg	eg	ee	hh		eh
9.37	2	aa	aa	i	aa	ii	fi	be	i		gi
12.96	3	ab[f]	aa		ab	i	fh	hh			i
16.56	4	be	ab		bc		hh	h			i
20.15	5	be	bb		bd			hh			
23.74	6	h	bd		cc						
27.34	7	h			dd						
30.93	8				e						
34.53	9										
38.12	10										
41.71	11										
45.31	12										
48.90	13										
52.50	14										
56.09	15										
59.68	16										

TABLE 4

Progressive Spoilage in Incubated Tubes of Meat Inoculated With 100 Spores per Gram Prior to Processing

Process at 115.6°C. (240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_2$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_3$, $NaNO_2$	Sugar, salt–1½, $NaNO_3$, $NaNO_2$
min.											
5.77	1	aa	aa		aa	bb	be	bb	hh	hi	ef
9.37	2	aa	aa		aa		bb	bc	i		ee
12.96	3	aa	aa	i	aa		ef	ei			hi
16.56	4	aa	aa		aa		ff	ee			gh
20.15	5	aa	aa		aa		h	gg			ii
23.74	6	aa	bb		bb		g	gi			ii
27.34	7	bf	bb	i	bb		h	gi			
30.93	8	hi	bd	ii	ee		h				
34.53	9		e		ee						
38.12	10				ei						
41.71	11										
45.31	12										
48.90	13										
52.50	14										
56.09	15										
59.68	16										

TABLE 5

Progressive Spoilage in Incubated Tubes of Meat Inoculated With 500 Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, $NaNO_3$, $NaNO_2$	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_3$, $NaNO_2$	Sugar, salt–1½, $NaNO_3$, $NaNO_2$
min.											
5.77	1	aa	aa	gg	aa	bb	cc	bb	gh	gh	ee
9.37	2	aa	aa	ee	aa	bb	ce	bb	hh		ee
12.96	3	aa	aa		aa		be	ce			fg
16.56	4	aa	aa		aa		ef	ee			hh
20.15	5	aa	aa		aa		gh	eg			hh
23.74	6	aa	aa		ab		gi	eg			hi
27.34	7	aa	ab		bb		gg	gh			hh
30.93	8	bf	bb		cd			h			h
34.53	9	ef	ee		cc						
38.12	10		ei		c						
41.71	11		e		ff						
45.31	12										
48.90	13										
52.50	14										
56.09	15										
59.68	16										

TABLE 6

Progressive Spoilage in Incubated Tubes of Meat Inoculated With 1,000 Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_3$	Sugar, $NaNO_2$, $NaNO_3$	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_3$, $NaNO_2$	Sugar, salt–1½, $NaNO_3$, $NaNO_2$
min.											
5.77	1	aa	aa	ee	aa	ee	ee	bb	hh	hi	ac
9.37	2	aa	aa	hh	aa	e	bb	be	hh		ee
12.96	3	aa	aa	h	aa		be	bb	hi		fg
16.56	4	aa	aa		aa		bb	bb			gh
20.15	5	aa	aa		aa		fg	ee			gi
23.74	6	aa	aa		aa		gg	ef			ii
27.34	7	aa	ab		bb		g	ff			
30.93	8	ae	bb		be		h	fg			
34.53	9	ee	ee		dd						h
38.12	10		ee		ce						
41.71	11		e		eg						
45.31	12										
48.90	13										
52.50	14										
56.09	15										
59.68	16										

TABLE 7

Progressive Spoilage in Incubated Tubes of Meat Inoculated With 10,000 Spores per Gram Prior to Processing

Process at 115.6°C.(240°F.)		Meat treated, 24 hours prior to processing, with—									
Time	Fo value	Nothing	Sugar	Sugar, salt–3½	Sugar, $NaNO_3$	Sugar, salt–3½, $NaNO_3$	Sugar, $NaNO_2$, $NaNO_2$	Sugar, $NaNO_2$	Sugar, salt–3½, $NaNO_2$	Sugar, salt–3½, $NaNO_3$, $NaNO_2$	Sugar, salt–1½, $NaNO_3$, $NaNO_2$
min.											
5.77	1	aa	aa	eg	aa	cc	bb	cc	hh	gg	bb
9.37	2	aa	aa	gg	aa	cc	bb	bc	hi	h	cd
12.96	3	aa	aa	hi	aa	cc	bc	bb			ee
16.56	4	aa	aa	i	aa		bg	cc			eg
20.15	5	aa	aa		aa		bg	cd			fg
23.74	6	aa	aa		aa		fg	de			gg
27.34	7	bb	ab		aa		hh	ee			hh
30.93	8	ab	bb		bb		gg	gg			
34.53	9	ac	bb		bc		hh	gg			
38.12	10	bc	bd		cc			i			
41.71	11		h		dd						i
45.31	12										
48.90	13										
52.50	14										
56.09	15										
59.68	16										

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CONTROL OF DUST CONTAMINATION IN AGAR POUR PLATE ANALYSIS OF MILK

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Many foods and drugs are subject to bacteriological analysis by the agar pour plate method of examination. Water, milk, and dairy products are but a few of the foods which are examined in this manner. Certain ampule preparations, medicinal pastes, and jellies are some of the drug products analyzed by estimating bacterial populations on agar pour plates. Control laboratories engaged in the examination of drug preparations requiring an agar pour plate have recognized the need of specially constructed cubicles or rooms provided with a constant supply of filtered air. The need for these and still other controls is self-evident in the examination of drug products where sterility is essential.

Although *Standard Methods of Water Analysis* (1936) and *Standard Methods for Examination of Dairy Products* (1941) are detailed in the specific requirements of analysis tending to uniformity and accuracy of results, neither of these recognized texts specifies the proper laboratory conditions under which analysis may be made. Precautions are reiterated throughout both volumes that media and laboratory equipment be protected against dust contamination following sterilization. It is recommended that each batch of media and dilution water be checked for sterility. Control measures are stipulated with a view to reducing air contamination to a minimum during analysis, but no specific requirements are set forth governing the size, construction, or care of the laboratory under which agar pour plate analysis can be carried out satisfactorily.

Black (1943), in a survey of milk laboratories throughout the country, recently found that the extent of violations occurring in milk examinations was sufficient to influence the accuracy of analysis. The data were obtained from observations of laboratory equipment, general preparation and sterilization of material, actual technique of making the analysis, and the manner of reading and recording of results. With specific reference to "Quarters and Facilities" he found that in general the quarters were adequate for the amount of milk examined. Failure to conform to some of the items listed under "Quarters and Facilities" had a direct relation to the accuracy of the results obtained. The author recommends that certain minimum standards of laboratory housing be included in *Standard Methods for the Examination of Dairy Products.*

EXPERIMENTAL PROCEDURE

A series of experiments was conducted, in the course of routine examination of milk and water samples, to determine the extent of air contamination during analysis.

The laboratory room, in which routine analyses were carried on customarily and in which part of these experiments were conducted, is 11 feet wide, 15 feet long, and 12 feet high, with one window and three doors. This room was not dustproof and was without benefit of any devices for maintaining air sterility. It contained the usual laboratory accommodations of work table, storage cabinets, incubators, centrifuge, steam and hot-air sterilizers, and a sink. An adjoining room, approximately equal in size, contained an old-fashioned, roll-top office desk and chair and an accumulation of cartons, milk bottles, and other articles. Except for the counting of agar plates on the office desk, this room was not used to any great extent in the examination of specimens.

The authors felt that these accommodations were inadequate for a sanitary milk and water laboratory. In their judgment six of the items listed under "Quarters" and five under "Facilities" [survey form for milk laboratories, page 5, used by Black (1943)] did not conform with recommended standards. Although the initial experiments were carried out in the laboratory rooms described above, these experiments were continued after changes had been made in the laboratory, devoting one room exclusively to analytical work and the adjoining room to dish-washing, media-making, and sterilization. The analytical laboratory was equipped with seamless table tops and dustproof cabinets. Such dust-catching objects as pictures, obsolete fixtures, and furniture were removed.

At the beginning of these experiments, which were carried out in the original laboratory described above, the walls, table, cabinets, and other surfaces were thoroughly washed and wiped down with 0.2 per cent Roccal (one ounce to four gallons of water). All the working surfaces were washed daily with this laboratory disinfectant prior to the start of the day's work. Control plates were poured daily for 10 days at the conclusion of the day's analysis of official milk specimens. In order to evaluate the need for the precautions noted above, a series of control plates was poured each morning before any clean-up was undertaken. After the rearranging and re-equipping of the laboratory, which enhanced and promoted cleanliness, these experiments were repeated.

To insure uniformity of manipulation of all control plates and to duplicate as nearly as possible routine analysis of milk, all experimental plates were seeded with one c.c. of sterile milk before plating with agar. These plates were subsequently incubated at 37.5°C.(99.5°F.) for 48 hours together with the routine official milk specimens.

RESULTS

Although the usual aseptic precautions were observed in making inoculations and pouring plates throughout these experiments, the results indicate a significant air-borne contamination when laboratory conditions are unsatisfactory. Over a period of four days and at a time of day when there was the least amount of disturbance to create air currents, 120 plates were inoculated and poured on working surfaces which were unwashed. The first series of plates showed 30 per cent contamination, whereas subsequent platings showed 13.3, 10, and 16.6 per cent contamination, with an average of 17.5 per cent for the total 120 plates examined (Table 1).

Sixteen plates contained only one colony, three plates contained two colonies, and two plates were contaminated with four colonies each.

To determine the effect of a disinfectant washing of dust-catching surfaces, the added aseptic precaution was observed of washing the table, ledge, and a portion of the wall with 0.2 per cent Roccal. An additional 130 plates were then seeded and poured under the identical laboratory conditions noted above. These plates were poured on four consecutive days following the analysis of official specimens. In this series 26.6 per cent of the plates were contaminated the first day; subsequent platings showed 13.3, 15.0 and 13.3 per cent contamination (Table 2); and 16.9 per cent of all the plates examined were contaminated. Eighteen plates contained only one colony and four plates contained two colonies each. Thus, in the total of 250 plates examined, with and without the precaution of a disinfectant wash, 82.8 per cent remained sterile and 17.2 per cent of the plates were contaminated with a maximum growth of four colonies.

Under conditions more conducive to cleanliness in the remodeled laboratory described above, 300 plates were poured and examined over a period of four days. These examinations were made following the analysis of official milk specimens. Prior to the start of the analysis all tables, shelves, and ledges in close proximity to the analytical table and all other surfaces where dust would readily accumulate were washed with the Roccal solution. Nine plates (3 per cent) were contaminated with one colony each. The remaining 291 plates (97 per cent) remained sterile. Twenty-seven unseeded plates, one for each bottle of agar used, likewise remained sterile at the end of the incubation period.

DISCUSSION

Although many investigators have placed considerable emphasis on the need for adequate facilities and quarters in which to conduct control laboratories, much of the criticism has been in the nature of conjecture. The results of this experiment indicate that the extent of error specifically due to air-borne contamination arising from inadequate facilities and quarters may be considerable. Even when aseptic precautions were rigidly observed and the table surfaces washed with a disinfectant, 16.9 per cent of the plates were contaminated. The accumulation of nondescript articles and the presence of obsolete fixtures and furniture all afforded places for the accumulation of dust. Because of lack of adequate storage facilities, many items of equipment were left exposed and these soon became dust-laden. These factors probably contributed toward contamination of the experimental plates.

Black (1943) refers to the psychological value of proper quarters and it seems reasonable to assume that under unfavorable laboratory conditions, as in the above case, little if any precautions are taken to reduce air-borne contamination to a minimum. We have observed that the room originally used as a laboratory was not conducive to cleanliness and therefore little cleaning was done, with a consequent heavy accumulation of dust on dust-catching surfaces.

After arranging a new laboratory provided with accessible and easy-to-clean facilities, considerably more pride was taken in keeping the prem-

TABLE 1

Air Contamination of Control Agar Pour Plates Made in Untreated Room

Number examined	Number sterile	Per cent sterile	Number contaminated	Per cent contaminated	Colony count
30	21	70.0	9	30.0	7 plates, 1 colony 1 plate, 2 colonies 1 plate, 4 colonies
30	26	86.7	4	13.3	3 plates, 1 colony 1 plate, 4 colonies
30	27	90.0	3	10.0	2 plates, 1 colony 1 plate, 2 colonies
30	25	83.4	5	16.6	4 plates, 1 colony 1 plate, 2 colonies

Total number of plates examined..... 120
Number of sterile plates..... 99
Per cent of sterile plates..... 82.5
Number of contaminated plates..... 21
Per cent of contaminated plates..... 17.5
Maximum number of developing colonies..... 4

TABLE 2

Air Contamination of Control Agar Pour Plates After Working Surfaces Were Washed With Disinfectant

Number examined	Number sterile	Per cent sterile	Number contaminated	Per cent contaminated	Colony count
30	22	73.4	8	26.6	6 plates, 1 colony 2 plates, 2 colonies
30	26	86.7	4	13.3	4 plates, 1 colony
40	34	85.0	6	15.0	5 plates, 1 colony 1 plate, 2 colonies
30	26	86.7	4	13.3	3 plates, 1 colony 1 plate, 1 colony

Total number of plates examined..... 130
Number of sterile plates..... 108
Per cent of sterile plates..... 83.1
Number of contaminated plates..... 22
Per cent of contaminated plates..... 16.9
Maximum number of developing colonies..... 2

ises on a high plane of cleanliness. The results of the change are reflected in reduction in the number of contaminants, from 17.2 per cent of the plates poured under unfavorable conditions to 3 per cent of those poured in a laboratory with adequate and improved facilities.

We are not passing judgment on the significance of the number of bacterial colonies which developed on the contaminated plates. It seems proper to assume that in laboratories with inadequate facilities and quarters fewer precautions might be taken than were observed in the course of these experiments, with a resultant increase in the number of bacterial contaminants.

SUMMARY

Two hundred and fifty experimental agar plates seeded with sterile milk and poured under unfavorable laboratory conditions showed 17.2 per cent contamination. Three hundred plates similarly seeded and poured in a laboratory with adequate facilities, which were conducive to cleanliness, showed 3 per cent contamination.

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CAROTENE AND CITRIN CONTENT OF PEAS AS INFLUENCED BY CHEMICAL TREATMENT

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While the effect of fertilizing treatment on vitamin content of plants is known, relatively little information exists concerning the influence of trace elements. In an early report, Honeywell and Dutcher (1930) stated that chlorotic spinach grown on soils deficient in manganese contained less vitamin A than normal spinach. Recently Lo and Wu (1943) found that the compounds of nickel, molybdenum, aluminum, zinc, copper, and iron so far studied have a physiological function of promoting vitamin P formation in mung-bean sprouts. Since it was not known whether the other parts of plants were similarly influenced, the present investigation, dealing with the effect of specific chemical treatment on the carotene and citrin contents of pea shoots and green peas by field experiment, was initiated. This seemed to be a worthy problem from both biochemical and practical standpoints.

EXPERIMENTAL PROCEDURE

One set of field experiments was laid out to test the effect of some specific chemicals (sulphates of nickel, aluminum, and zinc as well as molybdenum oxide) and a complete fertilizer (mixture of ammonium sulphate, bone meal, and straw-ash), singly and in combination. A native variety of pea (*Pisum sativum, L.*), namely Meitan Ying-Chia-Wan-Tou, was used as the experimental plant. The field experiment consisted of three blocks of plots laid out in randomized fashion, giving 10 treatments with three replicates. All plots were three and one-half by five feet with two rows of plants per plot. Each plot had six individual plants. The outside rows of each plot served as guard rows, preventing contamination of any plot by the chemicals and fertilizer used on adjacent plots. Application of the complete fertilizer (ammonium sulphate: bone meal: straw-ash = 3:1:4 by weight) was roughly such as would be 64 catties per mou before the plant seedlings were planted. All the specific chemicals were used in a concentration of 0.0001 N and applied in 50-c.c. portions per plant at 10-day intervals during the entire experimental period.

The method of carotene determination was that recommended by Hughes and Peterson, as reported by Munsey (1937), and the citrin was estimated by a modification of the Lorenz and Arnold (1941) method described in previous papers. Six determinations were made in all instances; results are given (Tables 1 2, 3, and 4).

DISCUSSION AND CONCLUSION

From an inspection of the above tables it is evident that there was a considerable variation in the carotene and citrin contents of pea shoots

and green peas treated with various compounds. The extent of this variation differed with the compound used. The compounds of nickel and zinc caused increase in both carotene and citrin of plants studied. The same is true in the case of molybdenum compound upon the citrin content. However, the aluminum compound did not seem to show a markedly favorable effect as compared with the control. This is due to the occurrence of abundant aluminum compounds in the soil, therefore no difference in citrin content between them was noticed. Moreover, it indicates the promoting effects were consistent in various parts of the plants—sprout, stem, leaf, and fruit.

The results obtained led to the conclusion that the compounds of nickel and zinc have a physiological function of promoting both carotene and citrin formation in plant metabolism. Similar conclusion was also reached in the case of molybdenum compound upon citrin formation.

The effect of the complete fertilizer (N, P, and K) was not consistent and significant, and no specific statement can be made, Lo and Chen (1943).

TABLE 1

Carotene Content of Pea Shoots as Influenced by Special Chemical Treatment (on Fresh Basis)

Treatment	Carotene content	Approximate vitamin A potency [1]	Relative value [2]
	γ/gm.	*I.U./gm.*	
O	46.16	76.93	100.00±4.12
C.F.[3]	48.03	80.05	104.05±1.66
$ZnSO_4$	50.27	83.78	108.90±4.21
$ZnSO_4$ + C.F.	49.04	81.73	106.24±4.87
$Al_2(SO_4)_3$	40.59	67.65	87.93±3.80
$Al_2(SO_4)_3$ + C.F.	40.00	66.67	86.66±4.87
$NiSO_4$	50.62	84.37	109.66±1.48
$NiSO_4$ + C.F.	58.45	97.42	126.62±3.05
MoO_3	39.89	66.48	86.42±5.33
MoO_3 + C.F.	40.00	66.67	86.66±2.56

[1] 0.6 was tentatively adopted as the conversion factor for transforming the number of γ of carotene into the number of International Units of vitamin A potency, Lo and Chen (in press). [2] Taking the sample of the control group ("O") as a standard with an arbitrary relative value of 100. [3] C. F. = complete fertilizer (N, P, and K).

SUMMARY

The effects of some specific chemicals (sulphates of nickel, aluminum, and zinc as well as molybdenum oxide) and a complete fertilizer (N, P, and K), singly and in combination, upon the carotene and citrin contents of pea shoots and green peas were studied by field experiment.

The results obtained led to the conclusion that the compounds of nickel and zinc have a physiological function of promoting both carotene and citrin formation in plant metabolism. Similar conclusion was also reached in the case of molybdenum compound upon citrin formation.

The effect of complete fertilizer (N, P, and K) was not consistent and significant.

TABLE 2

Carotene Content of Green Peas as Influenced by Specific Chemical Treatment (on Fresh Basis)

Treatment	Carotene content	Approximate vitamin A potency [1]	Relative value [2]
	γ/gm.	*I.U./gm.*	
O	7.47	12.45	100.00±3.70
C.F.[3]	7.05	11.75	94.38±1.21
$ZnSO_4$	13.15	21.92	176.04±3.51
$ZnSO_4$ + C.F.	12.97	21.62	173.62±4.00
$Al_2(SO_4)_3$	6.80	11.33	91.03±1.50
$Al_2(SO_4)_3$ + C.F.	6.70	11.17	89.69±3.90
$NiSO_4$	10.29	17.15	137.35±3.12
$NiSO_4$ + C.F.	9.05	15.05	121.15±4.10
MoO_3	7.63	12.72	102.14±1.25
MoO_3 + C.F.	7.25	12.10	98.04±4.45

[1] 0.6 was tentatively adopted as the conversion factor for transforming the number of γ of carotene into the number of International Units of vitamin A potency, Lo and Chen (in press). [2] Taking the sample of the control group ("O") as a standard with an arbitrary relative value of 100. [3] C. F. = complete fertilizer (N, P, and K).

TABLE 3

Citrin Content of Pea Shoots as Influenced by Specific Chemical Treatment (on Fresh Basis)

Treatment	Colorimeter reading [1]	Citrin content	Relative value
		mg./gm.	
O	4.466	1.353	100.00±3.92
C. F.	4.555	1.327	98.03±4.11
$ZnSO_4$	3.732	1.622	119.84±1.75
$ZnSO_4$ + C.F.	4.222	1.434	105.92±1.01
$Al_2(SO_4)_3$	3.579	1.690	124.86±1.42
$Al_2(SO_4)_3$ + C.F.	4.832	1.253	92.55±2.15
$NiSO_4$	3.687	1.640	121.14±6.03
$NiSO_4$ + C.F.	3.700	1.632	120.90±3.17
MoO_3	3.782	1.601	118.25±3.67
MoO_3 + C.F.	3.317	1.822	134.64±2.01

[1] Sample concentration: 0.2 gm. per c.c. iodine standard at 1 mm.

TABLE 4

Citrin Content of Green Peas as Influenced by Specific Chemical Treatment (on Fresh Basis)

Treatment	Colorimeter reading [1]	Citrin content	Relative value
		mg./gm.	
O	2.62	2.309	100.00±4.58
C. F.	2.45	2.469	106.93±5.28
$ZnSO_4$	2.00	3.025	131.01±6.42
$ZnSO_4$ + C.F.	2.00	3.025	131.01±5.21
$Al_2(SO_4)_3$	3.34	1.811	78.43±6.42
$Al_2(SO_4)_3$ + C.F.	3.09	1.958	84.80±5.09
$NiSO_4$	2.15	2.790	120.90±3.25
$NiSO_4$ + C.F.	2.17	2.788	120.75±3.15

[1] Sample concentration: 0.2 gm. per c.c. iodine standard at 1 mm.

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HISTOLOGICAL CHARACTERISTICS, TENDERNESS, AND DRIP LOSSES OF BEEF IN RELATION TO TEMPERATURE OF FREEZING

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As a result of earlier studies by two of the present authors and other workers, the fact seemed well established that the temperature of freezing has an important bearing on tenderness and on the drip loss of meat in thawing. To determine the basic cause of these effects was an obvious need. The purpose of the studies reported here was to throw light on the relation between the histological characteristics of beef frozen at different temperatures, on one hand, and tenderness and drip loss of the product, on the other.

REVIEW OF LITERATURE

In a study of ice formation, Moran (1931) found that in meat frozen at —20°C.(—4°F.) large crystals were formed in the center of the sample, whereas on the outside layer, where the time required for freezing was short, the ice crystals were very small. Moran (1932) also reported that approximately 82 per cent of the water in meat was frozen at —5°C. (23°F.). At lower temperatures more of the water was frozen, but it was of smaller percentage in relation to the reduction in temperature. Koonz and Ramsbottom (1939), using a modification of the Altmann-Gersh freezing-drying technic, studied frozen sections of chicken muscle and reported that ice crystals formed externally to the muscle fibers when freezing was done at —26.1°C.(—15°F.) or above. The ice formations became larger, fewer in number, and the component fibers and bundles of fibers were forced into compact groups when freezing was slow. At —75.6°C.(—104°F.) a sample several millimeters in thickness froze in a few seconds, and water separated from the fiber protoplasm so that the ice crystals were extremely small but numerous. On histological examination the fibers closely resembled those of fresh, unfrozen tissue. A whole bird frozen at —48.3°C.(—55°F.) showed a different picture, owing to slower freezing. The fibers appeared distorted and were characterized by jagged outer surfaces. The longitudinal sections of these fibers showed ice within the sarcolemma, which formed parallel to the long axis of the fiber, and extended its full length. The diameter of these ice columns increased in proportion to temperature increase.

DuBois, Tressler, and Fenton (1940, 1942) and Tressler and DuBois (1940) report that boned, rolled beef rib roasts, frozen in still air at —31.7 or —23.3°C.(—25 or —10°F.), or in an air blast at —17.8°C. (0°F.), showed some cell disruption, whereas those frozen in still air at —16.7°C.(2°F.) showed considerable breakdown in the tissue. The same conditions were observed in case of chicken flesh. Shrewsbury and co-

workers (1942), however, reported both intracellular and extracellular damage in pork muscle frozen at —26°F.(—32.2°C.).

Moulton (1929) in his book states that in slow freezing the muscle membrane may suffer a molecular or chemical breakdown and thus not be capable of reabsorbing the water during thawing. In fast freezing small ice crystals were formed and the tiny ice centers were dispersed throughout the muscle substance, being held apart by proteins and other cellular material, but there was no damage to cell walls. On thawing, the moisture was reabsorbed within the cell and no drip occurred.

It was shown by Hankins and Hiner (1938) that freezing at —6.7, —23.3, or —40°C.(20, —10, or —40°F.) increased the tenderness of beef as compared with that of beef held at 1.1°C.(34°F.). The greater change was found in those samples frozen at —10 and —40°F. Tressler, Birdseye, and Murray (1932) reported that quick-freezing of beef and subsequent storage at —18°C.(—0.4°F.) had a tenderizing effect. Paul and Child (1937) reported no tenderizing effect when beef was frozen at —19°C. (—2.2°F.). The work of Bray, Vail, and Mackintosh (1942) showed that freezing had no effect on tenderness of beef that had been aged for an unreported period of time before freezing, whereas Hiner and Hankins (1941) reported that beef samples stored at 0.6 to 1.7°C.(33 to 35°F.) for 5, 15, 25, and 35 days became more tender and that the tenderness was further increased by subsequent freezing, but to a decreasing degree with increasing storage time.

The proportion of drip loss in thawing has been found by several investigators to be related to temperature of freezing, slow freezing resulting in more and rapid freezing in less loss, Richardson and Scherubel (1908); Cool, Love, Vickery, and Young (1926); (unpublished results U. S. Dept. Agr.). Ramsbottom and Koonz (1939) found that when the area of exposed cut surface was small in relation to volume or weight of the sample of meat the quantity of drip was small irrespective of freezing temperature. In small steaks, where the exposed cut surface was large in relation to volume of meat, temperature was the significant factor, that is, the lower the temperature the less the drip. Empey (1933) reported that rate of freezing had no influence on drip but that the latter was a function of hydrogen-ion concentration. He found that the least drip came from muscle tissue having a pH value of 6.3 or higher. Sair and Cook (1938) found that the maximum drip occurred at a pH of 5.2, decreasing to no drip at pH 6.4. Also the time elapsing between slaughter and freezing was a factor—the longer the aging period the less was the drip. Ramsbottom and Koonz (1940) reported that drip decreased as time between slaughtering and freezing increased but found only a small change in pH after the first day.

EXPERIMENTAL PROCEDURE

Four pairs of short-loin cuts from Shorthorn steers produced and slaughtered at the Beltsville Research Center of the United States Department of Agriculture, Beltsville, Maryland, were used in this study. One steer was of beef type, another of dual-purpose type, and two were of dual-purpose crossed with beef type. The animals were fed individually

on a well-balanced ration and each was slaughtered when it had attained the weight of approximately 900 pounds. The average age of the steers when slaughtered was about 16 months. The carcasses varied in grade from low Choice to low Commercial, averaging Good. The separable fat in the rib varied from approximately 35 to 23.5 per cent, with an average of 30.5 per cent.

After each carcass was chilled for four days at approximately 0.6 to 1.7°C.(33 to 35°F.), the short loins were removed. On the fifth day after slaughter the longissimus dorsi muscle was removed from each short loin. Five pairs of cubical samples, approximately 1.5 inches on a side, were cut from each pair of muscles. Effort was made to cut parallel with the muscle-fiber bundles on the sides and at right angles on the ends. The samples were paired as rights and lefts and care was used not to freeze two samples from the same location at the same temperature. After cutting, the fresh samples were placed carefully on racks and frozen in air, without forced circulation, at —7.8, —17.8, —23.3, —40, or —81.1°C.(18, 0, —10, —40, or —114°F.). Freezing at —114°F. was accomplished by placing a layer of dry ice in a large vacuum bottle, then a covering of tin plate, the samples of meat, a covering of tin plate, and a thick layer of dry ice. The vacuum bottle was then placed in a —40°C.(—40°F.) freezer with the lid off. The samples were allowed to remain in the freezers for 24 hours before sectioning and photographing.

Obviously in a study of histological changes in frozen meat it is imperative that the sections removed for examination be as nearly identical to the whole frozen sample as possible. It is also desirable to have permanent sections representative of the frozen material for detailed study of the fibers themselves. Techniques used by other investigators were regarded as inadequate for the work reported here and therefore it was necessary first to develop suitable procedures. Various modifications in procedure were tried and the following frozen-section and mounting and staining methods were finally adopted and used.

Frozen-Section Method: A sliding microtome, precooled in a room maintained at approximately —7.8°C.(18°F.), was used to section the frozen blocks of meat. The blocks were so trimmed to fit the clamp that true longitudinal or cross sections of the muscle fibers could be obtained. Approximately one-eighth inch of meat was then cut from the top of the frozen sample to remove any tissue that had been subjected to surface drying. Sections 15 microns in thickness were then cut, carefully unrolled onto cold, clean slides, covered with cover slips, and examined microscopically. While the examination was carried out, the section was illuminated by means of a 200-watt, tungsten-filament lamp, used with a blue filter of high transmission at 4500 Å. The light was placed far enough from the reflecting mirror of the microscope to prevent the melting of the ice crystals. Both longitudinal and cross sections were examined and representative fields were photographed at magnifications of 150, 300, and 600 diameters, all in the same field.

To understand more clearly what takes place in the freezing of muscle it is well to consider the structure of a normal fiber. The individual

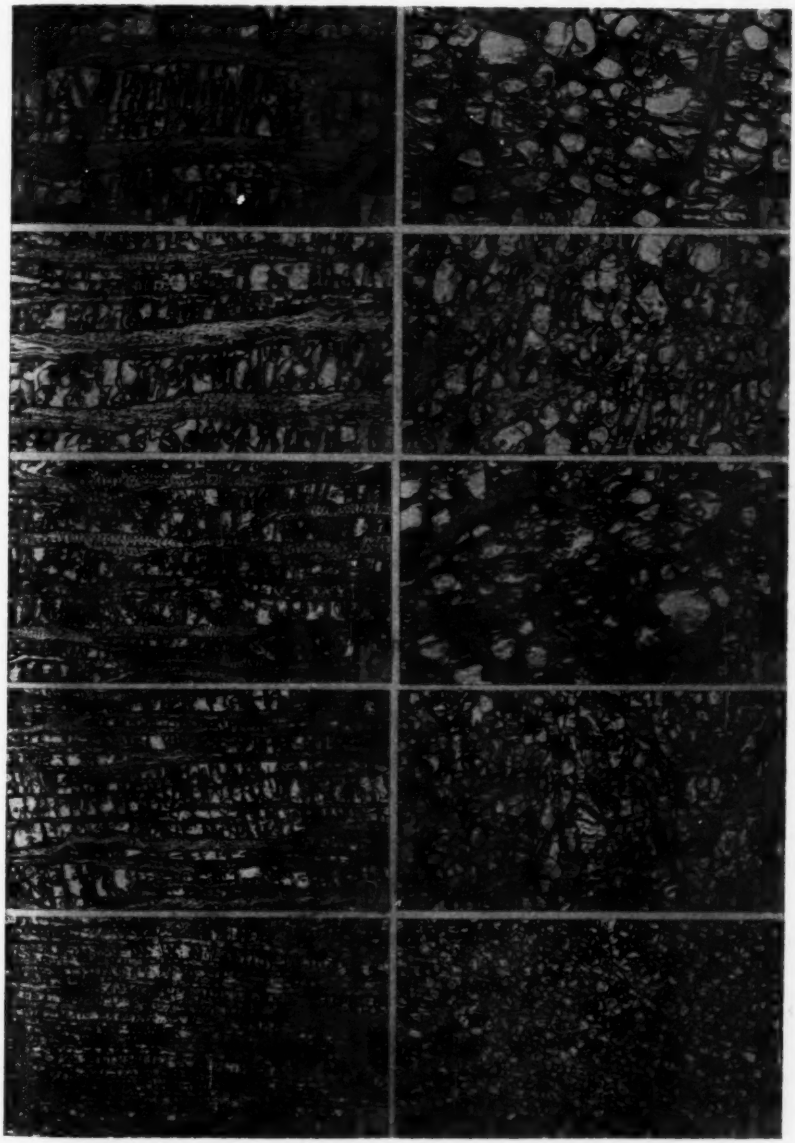

FIG. 1. Photomicrographs of longitudinal (on the left) and cross (on the right) sections of longissimus dorsi muscle of beef frozen at (A) 18°, (B) 0°, (C) —10°, (D) —40°, and (E) —114°F. (Original magnification × 150.)

muscle fibers that make up the voluntary muscle tend to be round or oval in cross section but variable in diameter and length. If the fiber is attached to the bone, the ends tend to be blunt; but if attached to other muscle fibers, they taper to a point. Each fiber is made up of contractile muscle substance enclosed in a tightly adherent extensible sheath, the sarcolemma. The muscle substance is a complex fluid containing water, proteins, fat-like substances, amino acids, salts, glycogen or breakdown products of glycogen such as lactic acid, and myofibrils, which are very fine fibrils that run parallel to one another longitudinally throughout the length of the fiber. Owing to light and dark bands or discs the muscle fiber has a striated appearance. Each muscle fiber contains numerous nuclei, usually located just beneath the sarcolemma.

Beef muscle contains approximately 70 to 75 per cent of water, most of it being within the fibers which are closely held together by interstitial connective tissue. A number of these fibers make up a muscle bundle and a number of the bundles in turn make up the entire muscle. Normally the muscle fibers run approximately parallel with each other and are rather evenly distributed.

When water freezes it tends to crystallize as pure water, pushing aside the substances that are dissolved or suspended in it. Fig. 1 shows, on the left, longitudinal sections and on the right, cross sections of beef frozen at various temperatures. The longitudinal section (Fig. 1A) shows that when this freezing took place slowly, at 18°F., ice crystals apparently formed between the muscle fibers and drew water to themselves from the meat juices within the fibers. The result was the formation of large interfibrillar crystals that pushed the semi-dehydrated muscle fibers into irregular groups. No intrafibrillar ice crystals are readily visible.

As the rate and degree of freezing increased, with decreased temperatures, there appears to have been less time or opportunity for the transfer of water into the spaces between the cells. According to Figs. 1B, 1C, 1D, and 1E, interfibrillar crystals became smaller and small crystals of ice appeared within the fibers. Beginning at —10°F. the proportion of ice frozen within the fiber was so large that the fiber itself was ruptured (Fig. 1C). This rupturing of fibers was regarded as attributable to to expansion of the cell moisture on freezing and it was more and more extensive as the temperature of freezing was lowered to —40°F. and —114°F. (Figs. 1D and 1E). At the latter temperature the intrafibrillar freezing of the water was so rapid that it appeared to have ruptured nearly every fiber.

The cross-section views (Fig. 1) are of the same sample of meat as the adjoining photomicrographs of the longitudinal sections and are of the same magnification, 150 diameters. Again the ice crystals decreased in size, became more irregular in shape, more uniform in distribution, and much more numerous as the freezing temperatures were lowered to —114°F.

For a more detailed study of the changes that took place within the fiber during freezing, photomicrographs of the longitudinal sections were made of magnifications of 300 diameters (Fig. 2, left-hand illustrations)

FIG. 2. Photomicrographs of longitudinal sections of longissimus dorsi muscle of beef frozen at (A) 18°, (B) 0°, (C) —10°, (D) —40°, and (E) —114°F. (Original magnification × 300 on the left and × 600 on the right.)

and 600 diameters (Fig. 2, right-hand illustrations). These photomicrographs confirm the former observations, except for the few ruptures seen in the sample frozen at 0°F. (Fig. 2B). Attention is called to the swelling that took place in the upper fiber in Fig. 2E, showing at 600 diameters the effects of freezing at —114°F. This was due to expansion of the intrafibrillar ice area present in a small section of the fiber that had been split from the whole fiber.

Staining and Mounting Method: Histological sections of frozen meats with the ice crystals present do not permit a close examination of the effects of freezing on the structure of the fibers. Such an examination can be made more readily in stained sections. Stained, mounted sections also provide a permanent record which the other method does not. Since it was not practicable to complete a thorough examination of the fibers and to operate the photographic equipment at a working temperature of 18°F., a rapid, reliable method for obtaining sections of meat that had been frozen and thawed, as well as checking the observations made of the frozen sections, was desirable. The following method was used, the sectioning being done at the same time as that for the study by the other method. In fact, the two sections used were cut adjacent to those previously discussed.

The technique consisted of cutting the section, unrolling it carefully on a cold slide, thawing by warming the slide from beneath with the operator's finger, and then placing the fixed sections directly in 70 per cent alcohol or passing the slides through graded alcohols of 30 and 50 per cent concentration, for about two hours in each, before placing in 70 per cent alcohol. The sections were allowed to remain in 70 per cent alcohol overnight, then stained with hematoxylin and eosin, and mounted in the usual manner. Alcohol fixation caused some shrinkage. In later work much less shrinkage was encountered when the sections were dipped in 30 per cent alcohol for three or four seconds to fix the tissue to the slide and then withdrawn and placed in 50 per cent dioxane for 24 hours and then in absolute dioxane for another 24-hour period. This procedure was followed by the usual staining and mounting technic and was believed to preserve more nearly the original structure of the fiber.

These sections were studied microscopically and photographs made of representative fields. A yellow-green filter was used.

Photomicrographs at 150 magnifications of representative longitudinal and cross sections of samples frozen at 18 to —114°F. are shown (Figs. 3A, B, C, D, and E). They bring out the fact that in freezing at 18°F. the fibers were bunched and, in longitudinal section, distorted. As temperatures were decreased and freezing became more rapid, there were fewer fibers per group and they were more nearly parallel. The cross-section views also show less bunching as rate of freezing increased. Evidence that precipitated muscle proteins were present at all temperatures is shown (Figs. 3 and 4). Although smaller in quantity at 18°F., the precipitated protein became progressively more abundant as freezing temperatures were lowered. Intrafibrillar ice areas, shown as clear spaces, appeared at 0°F. with some breaking of the sarcolemma as shown in both

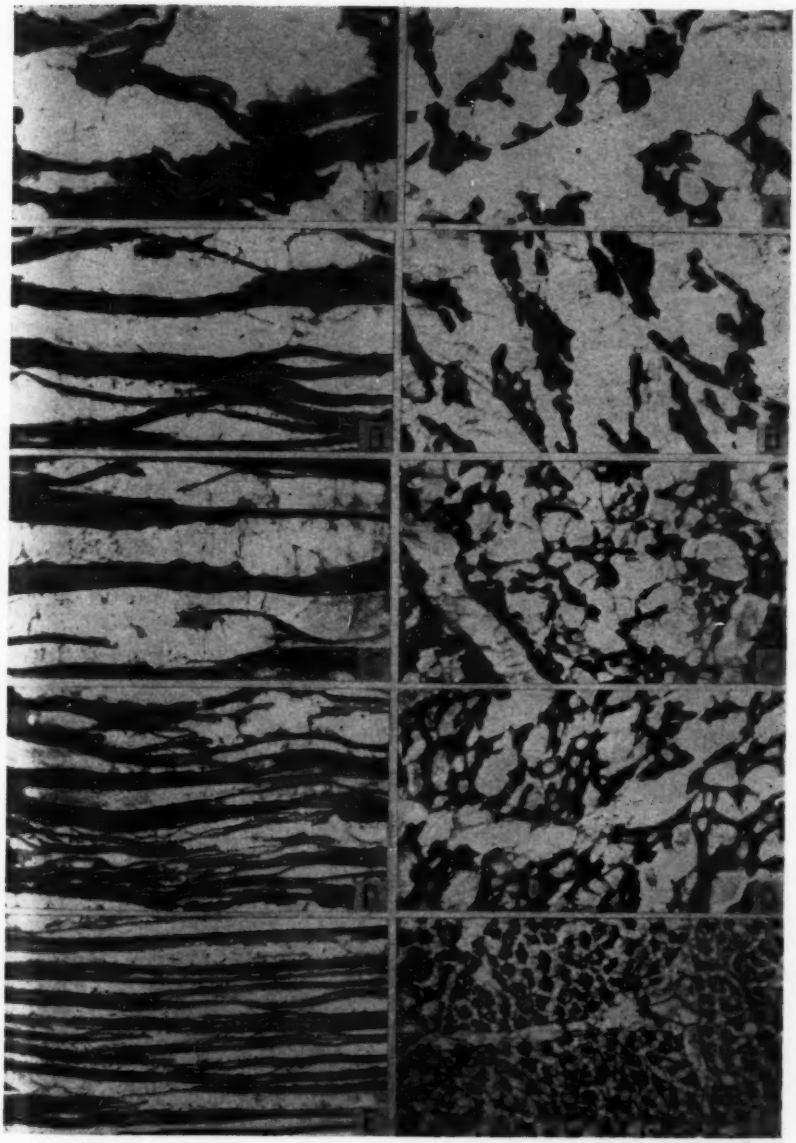

FIG. 3. Photomicrographs of longitudinal (on the left) and cross (on the right) sections of longissimus dorsi muscle of beef frozen at (A) 18°, (B) 0°, (C) —10°, (D) —40°, and (E) —114°F., after freezing, sectioning, fixing, and staining. (Original magnification × 150.)

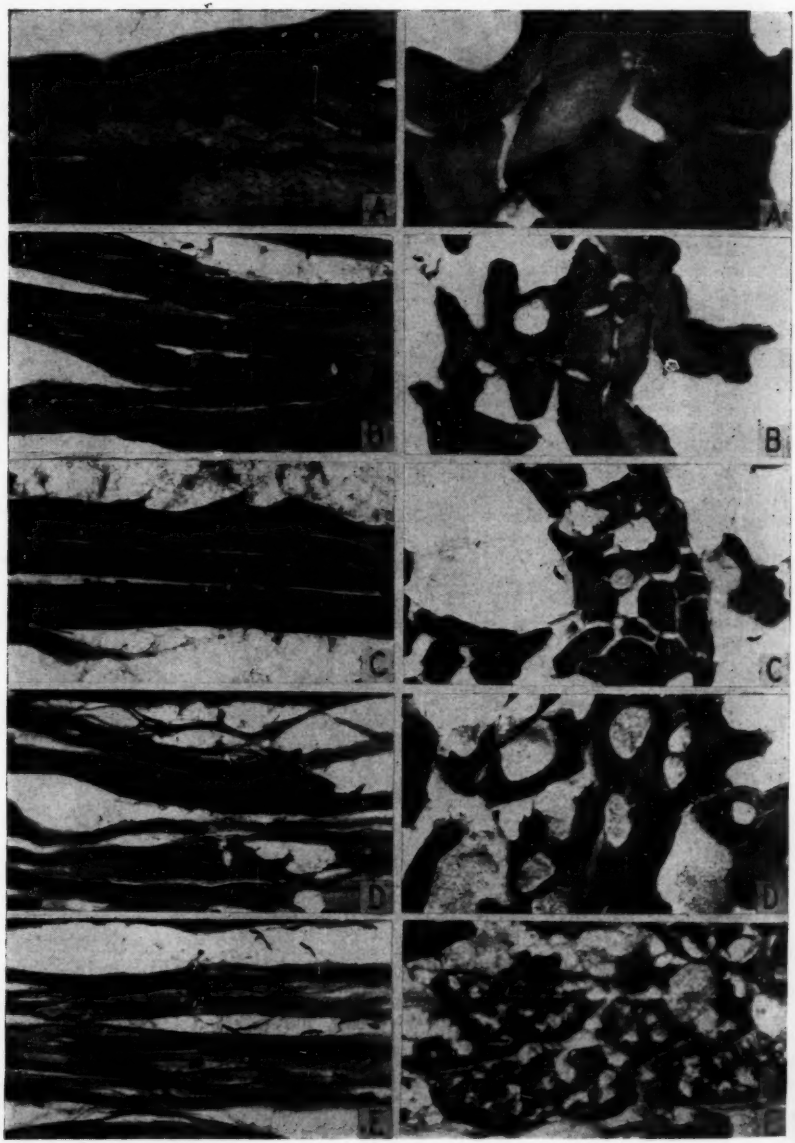

FIG. 4. Photomicrographs of longitudinal (on the left) and cross (on the right) sections of longissimus dorsi muscle of beef frozen at (A) 18°, (B) 0°, (C) —10°, (D) —40°, and (E) —114°F., after freezing, sectioning, fixing, and staining. (Original magnification × 600.)

the longitudinal and cross sections. This splitting became progressively more extensive as freezing temperatures were lowered to —114°F. Additional evidence of fiber splitting was the presence of a few nuclear fragments in the precipitated proteins at —10°F., with more as the temperature was decreased. It is of interest that there were few ice areas within the fibers, frozen at 0°F., as shown in the cross-section views. Those areas present were round and unaccompanied by fiber rupture. As the temperatures were lowered these small ice areas were found in a larger number of fibers and they became more and more irregular in shape. At —114°F. there appeared to be several ice areas within most of the fibers.

Photomicrographs taken at 600 magnifications (Figs. 4A, B, C, D, and E) show in more detail the changes brought about within the fiber by different temperatures of freezing. The indicated stretching, or the internal pressure to which the individual fibers were subjected at the low temperatures as compared with that at the higher temperatures, is of special interest.

After all sections were cut for the work discussed above, the remaining portion of each sample was defrosted at 34°F. (1.1°C.), fixed in 10 per cent formalin, dehydrated with dioxane, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for study. Microscopic examination of these sections showed little or no difference in structure, regardless of temperature of freezing. All sections appeared to have returned to approximately the normal condition found in the unfrozen, check section. The inadequacy of this method for determining the effects of different freezing temperatures on the structure of muscle tissue was indicated.

TENDERNESS AND DRIP LOSSES

For study in relation to the histological observations, it was considered desirable to determine the tenderness and drip losses from similar samples of beef frozen at the temperatures previously discussed, except that a temperature of —40°F. with an air blast of 1,516 cubic feet per minute was used in place of the 0°F. temperature previously discussed. From each pair of short loins from the four beef carcasses, six pairs of samples were cut, each sample being 1.5 inches thick. On the fifth day after slaughter 10 of the 12 samples were placed in their assigned freezers, according to a statistical plan, and the other two samples, used as unfrozen checks, were tested for tenderness the same day.

The samples remained in the freezers overnight, then were removed, weighed, and each was suspended over a large funnel inserted in a glass bottle. Thawing was done at 7.2 to 8.9°C.(45 to 48°F.). The samples remained at this temperature approximately 24 hours to become completely thawed. A weight was obtained of the drip that collected in the bottle and each sample was reweighed and tested for tenderness.

Tenderness determinations were made by the method outlined by Hankins and Hiner (1938), except for using the improved power-driven Warner-Bratzler shearing-strength machine. The shearing edge of the blade of this machine has the same degree of bluntness, but the opening in the blade is triangular instead of square.

During freezing the —40°C.(—40°F.) samples in still air and in an air blast had the lowest weight losses, whereas the —7.8°C.(18°F.) sample had distinctly the greatest loss (Table 1). There was little variation in percentage of weight loss during thawing. However, there was a distinct variation in the weight of drip collected during the thawing, the higher the temperature of freezing the greater being the weight of drip loss. With respect to weight of drip in relation to the total thawing loss, the greatest was at 18°F. and the least at —114°F. Those samples frozen at —40°F. in an air blast had a slightly lower proportion than similar samples frozen at —40°F. in still air. This probably was due to rate of freezing, less than two hours being required to freeze the former.

TABLE 1

Mean Weights of Fresh, Frozen, and Thawed Samples, Losses in Freezing and Thawing, and Resistance to Shearing (Tenderness) as Related to Temperature of Freezing

Temperature of freezing	Number of samples	Weight into freezer	Weight thawed	Weight loss in freezing	Weight of drip in thawing	Weight loss in thawing	Drip loss in relation to total thawing	Resistance to shearing (tenderness)	Decrease in resistance to shearing
		gm.	*gm.*	*pct.*	*gm.*	*pct.*	*pct.*	*lb.*	*pct.*
33-35°F.	8	641.3	.....	.....	.....	.....	.....	24.03	.....
18°F.	8	521.6	491.5	2.45	2.25	3.10	12.14	21.81	9.24
–10°F.	8	522.9	500.6	0.82	1.38	3.51	7.54	20.94	12.86
–40°F., still air	8	539.1	515.3	0.48	0.94	3.95	4.23	19.36	19.43
–40°F., air blast	8	543.5	520.9	0.43	0.75	3.73	3.75	18.30	23.85
–114°F.	8	527.6	503.4	0.92	0.38	3.53	1.91	17.17	28.55

From the results shown (Table 1) and the photomicrographs one may conclude that when beef was frozen at 18°F. the moisture froze largely outside or between the muscle fibers and, to a considerable extent, on thawing it was not reabsorbed but dripped from the meat, probably taking certain soluble constituents with it. On the other extreme, at —114°F., the moisture was very largely frozen within the fibers and was almost entirely reabsorbed by the fibers during the thawing. When the freezing was done at intermediate temperatures there were intermediate tendencies for reabsorption to occur.

The study of tenderness showed that as the temperature of freezing was decreased there was less and less resistance to shearing; for example, in the case of the beef frozen at 18°F. the resistance to shearing was 9.24 per cent less than in the case of the unfrozen, check samples. When the freezing temperature was —114°F. the resulting reduction was 28.55 per cent. It is evident from the relation between resistance to shearing and the histological characteristics reported above that much of the increase in tenderness of beef associated with freezing is the result of fiber splitting

and the breaking or stretching of the interstitial connective tissue surrounding the muscle fibers and fiber bundles.

SUMMARY AND CONCLUSIONS

Longissimus dorsi muscle of beef was frozen at 18, 0, —10, —40 and —114°F. in air without forced circulation, to investigate histological changes that occur with different rates of freezing. Samples were placed in their respective freezers for 24 hours, then sectioned, examined microscopically, and photomicrographs were made of representative fields.

Freezing at 18°F. resulted in the formation of large interfibrillar ice areas which pushed the fibers together into groups. No intrafibrillar ice crystals or areas were observed. Size of ice crystals and of ice areas between fibers decreased as freezing temperatures were lowered. Intrafibrillar freezing and some fiber-wall damage were visible at 0°F. and they became more extensive as temperature was further lowered. At the lowest temperature of —114°F. the fibers were even split longitudinally into several sections. Moreover, intrafibrillar ice crystals were very extensive and small.

Frozen sections were cut, placed on a cold slide, melted with the operator's finger, fixed in 70 per cent alcohol or graded alcohols and stained with hematoxylin and eosin. Observations confirmed changes noted in the study of the frozen section. Moreover, as freezing temperatures were lowered, precipitated proteins and nuclear fragments outside the fibers became more extensive. Intrafibrillar ice crystals and areas, with fiber splitting owing to ice formation, were clearly shown.

Dipping the slide in 30 per cent alcohol, after the section was thawed, was followed immediately by placing it in 50 per cent dioxane for 24 hours and absolute dioxane another 24 hours, then using the regular staining and mounting technic. This was believed to preserve more nearly the original structure of the fiber. Alcohol fixing solutions resulted in considerable shrinkage.

Frozen meat, thawed, fixed in formalin, dehydrated in dioxane, embedded in paraffin, sectioned and stained, did not preserve the detail observed by the previously described methods.

Beef samples aged five days, cut 1.5 inches thick and frozen at 18, —10, and —40°F. in still air; at —40°F. in an air blast; and at —114°F., increased consistently in tenderness as freezing temperature was lowered and time shortened. This increase in tenderness was interpreted as due to increased fiber rupture by intrafibrillar ice formation as well as to stretching and rupturing the interstitial connective tissue.

Drip during subsequent thawing decreased as freezing temperatures were lowered from 18 to —114°F. Increased intrafibrillar freezing and rupturing of fibers permitting the proteins to reabsorb a large proportion of the water originally frozen in the meat, were believed to be the cause.

This study included only meat frozen and examined within 24 to 36 hours. Further studies are needed to determine changes occurring during storage and the behavior of larger samples.

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THE B VITAMIN CONTENT OF RAW AND COOKED SWEET POTATOES

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Sweet potatoes are an important food in the South and appreciable amounts are consumed in other sections of the country. While considerable information is available on the carotene content—Fraps and Treichler (1933) and Swanson, Stevenson, Haber, and Nelson (1940)—and ascorbic acid content—Peterson, Sherwood, and Weaver (1944)—of sweet potatoes, only limited data are available for the B vitamins. Russell, Taylor, and Beuk (1943) reported three nicotinic acid values for sweet potatoes ranging from 5.3 to 6.3 μg. per gm. while Teply, Strong, and Elvehjem (1942) reported a single value of 12.9 μg. per gm. From the data of Cheldelin, Woods, and Williams (1943) on a single lot of sweet potatoes it has been calculated that they contained 0.41 μg. of riboflavin per gram of fresh potato. Information on the thiamin and pantothenic acid content of sweet potatoes is even more meager than for nicotinic acid and riboflavin. In this investigation we have assayed raw and cooked sweet potatoes for their thiamin, nicotinic acid, riboflavin, and pantothenic acid contents.

EXPERIMENTAL PROCEDURE

Material: Sweet potatoes were obtained from the Sweet Potato Investigations Laboratory at Gilmer, Texas; from the U.S.D.A. Research Center at Beltsville, Maryland; and from Opelousas, Louisiana. The varieties represented were Porto Rico, Nancy Hall, Triumph, and a strain designated as W. M. 3 which was developed from a white-skin mutation of the Porto Rico. The first two are deep yellow while the latter two are very light in color. Approximately 80 roots were used in the investigations.

Sampling and Cooking: A cork borer was used for cutting a core out of the potato for analysis. When cooking tests were to be made the sample was taken adjacent to the middle of the potato. The skin was cut from each end of the core so as not to include it in the sample used for analysis. The holes were tightly corked to prevent an uneven gain or loss of moisture from the cut surface of the potato during cooking. The core was shredded by means of a household grater, and samples were weighed for analysis.

The two methods of cooking used were baking and boiling. Both the baked and the boiled potatoes were cooked with their skins on. The baked potatoes were cooked in an oven at 204.4°C.(400°F.). The boiled potatoes were cooked in a covered container. They were not placed in the container until the water had come to a boil.

Methods of Analysis: After weighing, the samples for analysis were ground in a mortar and then transferred to Erlenmeyer flasks for autoclaving. Thiamin was determined by the fungus-growth method as de-

scribed by Hamner, Stewart, and Matrone (1943). Riboflavin was determined by the microbiological method of Snell and Strong (1939) on the autoclaved sample following filtration at a pH of approximately 4.6 to remove foreign growth stimulants.

Nicotinic acid was determined by the microbiological method of Krehl, Strong, and Elvehjem (1943) with the modification that the ground sample was suspended in 0.1 N hydrochloric acid rather than in N hydrochloric acid. This permitted the thiamin, riboflavin, and nicotinic acid assays to be made on the same hydrolyzate. Prior to adopting 0.1 N hydrochloric acid as a routine for the liberation of nicotinic acid, tests were made using 0.5 N sodium hydroxide, 0.1 N and one N hydrochloric acid. On sweet potatoes the nicotinic acid values were virtually identical with filtrate prepared by using 0.5 N sodium hydroxide, 0.1 N and one N hydrochloric acid.

Pantothenic acid was determined using the basal medium described by Neal and Strong (1943). A one-gram sample of the ground sweet potato was suspended in 50 ml. of sodium acetate buffer at a pH of 4.6 and autoclaved for about 30 minutes at 15 pounds pressure. After cooling, takadiastase and papain were added and enzymatic digestion carried out essentially as described by Cheldelin, Eppright, Snell, and Guirard (1942). The enzymatic treatment was considered essential as it was observed in preliminary trials that the pantothenic acid was not completely liberated from the raw sweet potatoes by autoclaving in water.

RESULTS AND DISCUSSION

Effect of Variety: Eight roots from each of four varieties of sweet potatoes were assayed for each of the four vitamins being studied. Only in the case of nicotinic acid was there any appreciable varietal difference in vitamin content. The average nicotinic acid values expressed in μg. per gm. were Nancy Hall, 5.40; Porto Rico, 5.16; W. M. 3, 3.56; and Triumph, 4.07. The first two varieties are a deep yellow in color while the last two are a very pale yellow or almost white. The varietal data for the other vitamins were omitted to conserve space since they showed no essential difference.

The data, while limited, did not indicate any significant differences in the B vitamins in sweet potatoes secured from three different states.

Values for Raw Sweet Potatoes: The average values and the ranges for thiamin, riboflavin, nicotinic acid, and pantothenic acid are summarized (Table 1). The average riboflavin value of 0.46 μg. per gm. agrees quite well with the value of 0.41 μg. per gm. reported by Cheldelin, Woods, and Williams (1943). The average nicotinic acid value of 5.56 μg. per gm. is in very good agreement with the values reported by Russell, Taylor, and Beuk (1943). Most of the values included (Table 1) are from potatoes of the Porto Rico variety. Hence, any slight varietal differences are, in the main, excluded from the average value for nicotinic acid. Our average value of 1.39 μg. per gm. of thiamin is of the same order as the single value of 1.09 μg. per gm. for sweet potatoes reported by Lane, Johnson, and Williams (1942). It is of interest to note that the thiamin values for sweet potatoes are of the same order as many of

the thiamin values reported by Meiklejohn (1943) for Irish potatoes. The pantothenic acid content of raw sweet potatoes averaged 10.95 μg. per gm. and ranged from 8.01 μg. to 13.70 μg. per gm.

Effect of Cooking: In the cooking tests a sample was taken out with a cork borer. The potato was then weighed. The holes made by removing the sample were plugged by means of corks. After cooking, the potatoes were again weighed. In calculating the retention of the vitamins, the values per gram were multiplied by the total weight of the potato before and after cooking so as to account for any change in weight.

TABLE 1

Vitamin Content of Raw Sweet Potatoes[1]

	Thiamin	Riboflavin	Nicotinic acid	Pantothenic acid
	μg./gm.	μg./gm.	μg./gm.	μg./gm.
Average	1.39	0.46	5.56	10.95
Range	1.00-1.70	0.35-0.60	4.00-6.80	8.00-13.70

[1] Values for 40 potatoes.

On baking, sweet potatoes were found to lose an average of 16.7 per cent of their weight, while those cooked by boiling in water gained an average of 4.9 per cent in weight. This loss or gain in weight by the respective methods of cooking was definitely correlated with the retention of the vitamins studied. Baked sweet potatoes retained an average of 75.5 per cent of their original thiamin, while boiled sweet potatoes retained an average of 92.3 per cent. Our minimum value of 62.4 per cent retention for thiamin in baked sweet potatoes is much larger than the single figure of 25 per cent reported by Lane, Johnson, and Williams (1942).

Retention of riboflavin averaged 88.6 per cent for the baked potatoes and 103.2 per cent for the potatoes cooked in boiling water. The figure for baked sweet potatoes is in accord with the 13 per cent loss reported for riboflavin by Cheldelin, Woods, and Williams (1943).

Retention of nicotinic acid in the sweet potatoes cooked by baking averaged 85.1 per cent, while those cooked in boiling water averaged 100.6 per cent. These figures differ somewhat from 95.2 per cent retained in a single lot of baked sweet potatoes by Cheldelin, Woods, and Williams (1943) and values ranging from 85.2 per cent to 97.7 per cent for three lots of sweet potatoes cooked by boiling as reported by Russell, Taylor, and Beuk (1943).

The retention of pantothenic acid averaged 76.8 per cent for the baked sweet potatoes and 99.9 per cent for the potatoes cooked by boiling in water. While no other data are available for the retention of pantothenic acid in sweet potatoes, the greater loss of pantothenic acid during baking is in accord with the observations of Cover, McLaren, and Pearson (1944) in well-done roast beef where the per cent of pantothenic acid retained was less than in the case of riboflavin and nicotinic acid.

The data (Table 2) leave little doubt as to the efficacy of boiling over baking in retaining the B vitamins during cooking.

Sprouts: A limited amount of information was secured on the vitamin content of sweet-potato sprouts and on sweet potatoes after sprouting. Roots were placed at a depth of about eight inches in clean sand. They were kept moist and the sprouts allowed to grow until they just penetrated the surface. Both the sprouts and sprouted potatoes were assayed for thiamin, riboflavin, nicotinic acid, and pantothenic acid.

TABLE 2

Retention of Vitamins in Cooked Sweet Potatoes

Method of cooking	Number of potatoes	Cooked wt. as pct. of original wt.	Thiamin	Riboflavin	Nicotinic acid	Pantothenic acid
Baked			*pct.*	*pct.*	*pct.*	*pct.*
Average	20	83.3	75.5	88.6	85.1	76.8
Range	....	70.3-88.8	62.4-93.9	73.9-102.7	70.9-106.6	68.8-96.4
Boiled						
Average	14	104.9	92.3	103.2	100.6	99.9
Range	....	100.0-112.0	80.7-102.7	89.0-113.5	86.8-108.9	77.6-108.5

There was considerable variation in the thiamin and nicotinic acid values in both the sprouts and potatoes after sprouting. With the exception of thiamin the riboflavin, nicotinic acid, and pantothenic acid contents of the sprouts and sprouted potatoes appear to be essentially as high as in the unsprouted potatoes (compare with figures in Table 1). Since the weight of the potato plus the sprouts exceeded the weight of the root at the time of planting, it suggests that these vitamins were synthesized in the absence of light. The thiamin values are in general lower than those reported in Table 1. The wide variation and limited number of observations make any further interpretation of doubtful value.

TABLE 3

Vitamin Content of Sprouts and Sprouted Sweet Potatoes

Root No.	Potatoes				Sprouts			
	Thiamin	Riboflavin	Nicotinic acid	Pantothenic acid	Thiamin	Riboflavin	Nicotinic acid	Pantothenic acid
	μg./gm.	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*
1	1.22	.57	2.2	11.5	1.27	.64	4.4	10.0
2	0.53	.45	5.1	10.5	0.60	.57	6.1	10.0
3	0.62	.51	4.0	11.2	0.65	.57	6.0	11.2
4	0.61	.48	4.8	10.0	0.57	.48	4.8	11.1
5	0.35	.45	5.0	11.1	0.90	.49	5.4	12.0
Av.	0.67	.49	4.2	10.9	0.80	.55	5.3	10.9

SUMMARY

The thiamin, riboflavin, nicotinic acid, and pantothenic acid contents of raw and cooked sweet potatoes are reported.

Sweet potatoes cooked by baking retained 75.5 per cent of the thiamin, 88.6 per cent of the riboflavin, 85.1 per cent of the nicotinic acid, and 76.8 per cent of the pantothenic acid. There was less loss of the vitamins when the potatoes were cooked by boiling. Boiled sweet potatoes retained 92.3

per cent of the thiamin, 103.2 per cent of the riboflavin, 100.6 per cent of the nicotinic acid, and 99.9 per cent of the pantothenic acid.

The riboflavin, nicotinic acid, and pantothenic acid contents of sprouts and the sprouted sweet potatoes were essentially as high as for unsprouted potatoes, while for thiamin the values were somewhat lower.

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DEGREE OF PIGMENTATION AND ITS PROBABLE RELATIONSHIP TO INVERTIVE ACTIVITY OF HONEY[1]

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It is generally well known that enzyme action is the key to those biological activities of the bee with which it converts to honey nectar gathered from the flowers. Perhaps not so well known, however, is the fact that honey contains enzymes and that some of them have a technological, others an analytical, interest. Diastase and invertase are representative of the former; catalase and diastase are representative of the latter in the sense that they have been suggested as the point of departure in the development of methods for distinguishing either between genuine and artificial honeys or heated and unheated ones.

An interest in the enzymes of honey probably first developed about 70 years ago. It has not, however, yielded a rich literature,[2] yet in it are revealed two lines of interest with respect to honey invertase. On the quantitative side are studies of its invertive ability; on the qualitative side are several communications, some of which center around the question of origins of the invertase and others around the thesis that its properties are not like those of yeast invertase.

Earlier workers in the former field include Langer (1909) and Achert (1912), who followed polariscopically the hydrolytic action of honey upon standardized sucrose solutions; and Auzinger (1910) and von Fellenberg (1911), who used Fehling's solution for the same purpose. The invertive power of a number of honeys was determined in the present study by means of the blue color produced by the reducing action of invert sugar on an acid molybdate solution, adapting to our needs the Baier-Neumann (1908) test for added sucrose in milk.

EXPERIMENTAL PROCEDURE

The 23 honeys used in this study represent 13 known floral sources plus three market samples of unknown history. Except for the latter, all were obtained from apiarists in eight states.

Experimental details were developed in the light of the researches of Nelson and Cohn (1924) on honey invertase and in consideration of the average composition of honey, viz., approximately 40 per cent levulose, 35 per cent dextrose, less than one per cent sucrose, 17.5 per cent water, and small amounts of ash and free acids.

Invertive activity was measured, under sharply defined experimental conditions, by noting the change in the invert sugar content of the honey

[1] Published here by permission of *Industrial and Engineering Chemistry.*

[2] For literature reviews on this subject see papers by Moureau [Ann. fals. 4 (1911), 65]; and Gothe [*Z. Untersuch. Nahr. Genussm.* 28, (1914), 273].

solution (25 gm. in 100 ml.) six hours after the addition of four grams of sucrose. Because exploratory experiments had shown that the reagents exerted no detectable hydrolytic effect upon the added sucrose, it was concluded that the sole cause of inversion was the invertase of the honey under examination, thus making corrections for a blank determination unnecessary.

Hydrogen-ion concentrations and color comparisons were made, respectively, with pH meter and photoelectric colorimeter. The latter was standardized against hydrolyzed saccharose solutions, a straight-line graph resulting when the log of per cent transmission was plotted against concentration.

Inasmuch as the following procedure had been found to yield working quantities of reducing sugar in a minimum reaction period, it was used throughout this study. The pH of a 25-gram sample of honey, dissolved in 40 c.c. of water in a 100-c.c. volumetric flask, was adjusted to 5.97 $\pm$ 0.5 with .02 *N* sodium hydroxide solution, after which there were added potassium acid phthalate buffer solution (25 c.c.) and, before dilution to the mark, four grams of sucrose. The flask was then put into a 40°C. (104°F.) bath where it was brought to temperature (five min.) before a one-c.c. portion was pipetted, in triplicate, into a 25-c.c. colorimeter tube. The addition of 10 c.c. of a four-per cent ammonium molybdate solution and 0.25 c.c. of a 4 *N* nitric acid followed. At one-minute intervals each tube, its contents well stirred, was then placed for 2.5 minutes in a constant-temperature water bath at 65°C.(149°F.) whereupon, in turn, it was immersed in an ice bath for three minutes, let stand at room temperature for seven minutes, filled to the mark, and again let stand. Eight minutes thereafter its reducing sugar was determined with the aid of an Evelyn colorimeter fitted with a 660 filter.

At the end of six hours, triplicate one-c.c. portions were again removed from the original honey solution—its temperature in the meantime had been maintained at 40°C.(104°F.)—and the reducing sugar content again determined. The difference in values before and after hydrolysis, as read from a standard graph in which the log of galvanometer readings had been plotted against milligrams of fructose, represented the amount of reducing sugar formed as fructose. From this value the invertive activity, or milligrams of sucrose hydrolyzed by one gram of honey, was calculated.

RESULTS AND DISCUSSION

Data for each of the samples analyzed during the course of this survey have not been made a part of this record, because primarily the center of interest was the degree of pigmentation rather than the floral source of the honey. This emphasis is consistent with current merchandizing practices. According to its position on the Pfund scale—Sechrist (1925)—each honey was given a "light" or "dark" classification as typifying, in a qualitative sense, the most obvious difference in pigmentation. The effect of this division was the assignment of those grading water-white, white, and extra light amber to the first group (Table 1); light amber, amber, and dark to the second (Table 2).

TABLE 1

Invertive Activity of Light Honeys

Predominating floral source	Origin	Color	Invertive activity
White sage	California	Water-white	9
Orange blossom	California	Water-white	10
White clover	Wisconsin	White	30
White clover	New York	White	33
Tupelo	Florida	Extra light amber	19
White sage	California	Extra light amber	34
Unknown	Unknown	Extra light amber	39
Horsemint	Texas	Extra light amber	70

TABLE 2

Invertive Activity of Dark Honeys

Predominating floral source	Origin	Color	Invertive activity
Alfalfa	California	Light amber	54
Sage	California	Light amber	60
Thistle	California	Light amber	69
Holly blossom	Arkansas	Light amber	70
Tupelo	Florida	Light amber	105
Clover	Illinois	Light amber	74
Mexican clover	Georgia	Light amber	90
Titi	Georgia	Light amber	74
Tupelo	Georgia	Light amber	62
Buckwheat	New York	Amber	130
Tamarisk	California	Amber	142
Spikeweed	California	Amber	158
Wonder honey plant	Georgia	Amber	90
Unknown	West Indies	Amber	87
Unknown	Unknown	Dark	170

TABLE 3

Color Grade of Honey in Relation to Invertive Activity

Color grade	Number of honeys examined	Pfund scale reading	Invertive activity
Light honeys			
Water-white	2	1.1	9
White	2	1.9	31
Extra light amber	4	4.5	40
Average	....	....	27
Dark honeys			
Light amber	9	6.3	73
Amber	5	9.5	152
Dark	1	12.0	170
Average	....	....	132

If the data (Table 3) are plotted, a smooth curve results. That it has quantitative significance is problematical. Qualitatively, however, one interpretation appears to be obvious: the darker the honey the greater its invertive activity towards sucrose. This observation parallels one previously made—Schuette and Pearlstein (1938)—with respect to diastase. The differences in this activity, although distinct, do not increase rapidly in the lower color grades but from this point on they move apart with large strides.

Although a fact-finding survey as to the relative powers of dark and light honeys to hydrolyze sucrose solutions may not in itself be important, still the study has interest in that it furnished the stimulus to develop a technique for measuring the activity of invertase. Perhaps in the hands of others, when adapted to their particular needs, it may become another tool in quantitative biochemical analysis.

SUMMARY

Invertive activity, measured by noting the change in the invert sugar content of a honey solution of controlled pH six hours after the addition of sucrose and expressed as milligrams of sucrose hydrolyzed by one gram, apparently bears a qualitative rather than a quantitative relationship to the degree of pigmentation (Pfund scale) of honey. Dark honeys were found to exceed the light ones in this property.

Using the photoelectric colorimeter and an adaption of the old Baier-Neumann test for added sugar in milk, the following average values were found: for the light honeys (water-white, white, and extra light amber) 27; for the dark honeys (light amber, amber, and dark) 132.

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STABILITY OF B VITAMINS IN GRAPE JUICES AND WINES[1]

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The natural vitamin content of grape juices and of the wines made from them is measurable but not large, Morgan, Nobles, Wiens, Marsh, and Winkler (1939). The problem of prevention and cure of the avitaminoses sometimes developed by habitual drinkers of distilled liquors and, less often, of wines has been given attention in recent years. Some of the most severe symptoms of acute alcoholism have been ascribed to certain B-vitamin deficiencies, particularly to thiamin deficiency, Jolliffe (1940). The clinical manifestations of chronic alcoholism of wine drinkers are usually different from those of distilled liquor drinkers. The latter often present the nervous and cardiac symptoms of beri-beri, the former the dermatitis, mental confusion, and digestive disorders of pellagra. The presence of small amounts of different members of the B-vitamin complex in these different classes of beverages may account for the variety of symptoms.

If the consumption of calories from alcohol increased the thiamin or other B-vitamin requirement as does the consumption of carbohydrate, the provision of enough B vitamins in the wine to meet the need is desirable. If added synthetic vitamins are stable in wines and grape juices, such fortification might be economical and justifiable. Moreover the use of wines as carriers of vitamins for medicinal use may in some cases be desirable if such mixtures prove to be stable.

EXPERIMENTAL PROCEDURE

To test the stability of added B vitamins in wines two lots of grape juices and wines[2] were prepared in 1940 and 1941, and in each case part of each lot was fortified with a solution containing a mixture of crystalline vitamins. In the 1940 experiment the juice and wines had added to them one milligram per liter each of thiamin hydrochloride, pyridoxine hydrochloride, and riboflavin, and 10 milligrams per liter each of nicotinic acid and calcium pantothenate. The 1940 wines were made from Burger grapes and the 1941 from Tokays. In the latter case the juice and wine had added to them two mg. per liter each of thiamin hydrochloride and calcium pantothenate, one mg. per liter each of pyridoxine and riboflavin, and 10 mg. of nicotinic acid. The 1940 lot of samples were examined, shortly after the wines were made, for thiamin, pantothenic acid, pyridoxine, riboflavin, and pantothenic acid and were re-examined at intervals for more than a year after storage under various conditions. In addition

[1] Assistance was rendered this study by the California Wine Institute.

[2] The juices and wines were prepared by A. J. Winkler of the Division of Viticulture and G. L. Marsh of the Fruit Products Division.

several commercially produced wines of different types and of known history were assayed for riboflavin and pantothenic acid.

Thiamin was determined by a rat-growth method in which the growth of a series of animals fed graded amounts of pure thiamin chloride, three, five, and eight micrograms daily, was used to construct a curve upon which the thiamin value of the unknown could be read from the growth induced in similar rats fed the same thiamin-free basal diet and a known amount of the juice or wine to be assayed.

The basal diet consisted of washed casein 22, hydrogenated cottonseed oil 9, salt mixture [Hubbell, Mendel, and Wakeman (1937)] 2.5, sucrose 66.5. The vitamin supplements fed separately were per rat per day in micrograms—riboflavin 20, pyridoxine 20, and calcium pantothenate 120; five mg. choline chloride; and gray fish liver oil to provide 143 I. U. of vitamin A and 10 I. U. of vitamin D. After two to three weeks' depletion on this diet the rats were given either the crystalline thiamin chloride or the grape juices or wine and their growth was noted for 28 days. The vitamin-reinforced samples were fed at the level of three c.c. per day and the unreinforced, 10 c.c. per day. Two groups of thiamin-fed controls were used, one receiving no alcohol to compare with the juice-fed animals and one receiving three or 10 c.c. of 12-per cent ethanol solution daily to compare with those receiving wine.

In this and in the riboflavin and pyridoxine assays which were made by the same method it was found that the control groups which received the alcohol, both with and without thiamin, made better growth or survived longer on the deficiency than did those which received no alcohol. This is in accord with earlier observations by Morgan *et al.* (1939) and with the resistance to the neuropathy of thiamin deficiency shown by alcohol-fed rats, Lowry, Sebrell, Daft, and Ashburn (1942). This does not support the theory that alcohol metabolism involves the use of thiamin.

The thiochrome method of Conner and Straub (1941) was also used.

Riboflavin was determined by a rat-growth method similar to that used for thiamin and also by the microbiological method of Snell and Strong (1939).

Pyridoxine was determined by a similar rat-growth method and attempts were made to use a modification of the chemical method of Scudi (1941). The rat-growth method was essentially like that which Clarke and Lechycka (1943) have shown to be reliable.

Pantothenic acid was determined by the microbiological method of Strong, Feeney, and Earle (1941).

The pasteurized juices and wines were stored in glass bottles at room temperature of 15.6 to 21.1°C.(60 to 70°F.) or in the refrigerator at 4.4 to 10°C.(40 to 50°F.), as indicated in the tables.

The samples prepared for the study of the effect of light on riboflavin were kept on a high shelf in the laboratory exposed to strong afternoon light and at a temperature of 60 to 70°F.

DISCUSSION OF RESULTS

Thiamin: Burger juices and wines retained both the natural and added thiamin in inverse proportion to the amount of SO_2 used and retained

(Table 1). The loss of thiamin appeared to be governed by the amount of free rather than total SO_2 present. Thus Wine 2988 with 37 p.p.m. of free SO_2 lost more thiamin than did No. 2990 with 21 p.p.m. although the latter had the larger total SO_2. The original wines contained little or no thiamin, at best one-half or one-fourth that contained in the juice. The fortified juice and wines retained the added thiamin well and generally better in the wines having the lower SO_2 content.

TABLE 1

Thiamin Content of Burger Grape Juices and Wines as Affected by SO_2 and Thiamin Addition, Three Months After Vinification, Stored at 7.2°C.(45°F.)

Sample	Description	SO_2		Thiamin		Added thiamin retained
		Free	Total	By rat-growth method	By thiochrome method	
		p.p.m.	*p.p.m.*	*μg./100 gm.*	*μg./100 gm.*	*pct.*
Juice A	Burger grapes, juice pressed and pasteurized 30 min. at 76.7°C.(170°F.)	....	....	49	72	....
Juice B	Same as Juice A with addition per liter of 1 mg. each thiamin, riboflavin, and pyridoxine and 10 mg. each nicotinic acid and calcium pantothenate	....	....	140	120	91
Wine No. 2988A	Made with 1% pure yeast, no SO_2 before fermentation, 75 p.p.m. after fermentation was complete	37	62	0	....	....
2988B	Same as 2988A with vitamins added as in Juice B, after vinification	37	62	69	68	69
2989A	100 p.p.m. SO_2 immediately after pressing and before fermentation	18	58	24	....	....
2989B	Same as 2989A with vitamins added as in 2988B	18	58	109	108	85
2990A	250 p.p.m. SO_2 before fermentation	21	86	0	....	....
2990B	Same as 2990A with vitamins added as in 2988B	21	86	100	96	100
2991A	No SO_2	5	8	13	....	....
2991B	Same as 2991A with vitamins added as in 2988B	5	8	106	133	93

The Burger juice had about the same thiamin value as the juices of other varieties of grapes examined previously, 49 as compared with the range of 30 to 57 micrograms per 100 ml., Morgan *et al.* (1939). The values found in the wines were also similar to those of the earlier study.

The agreement between the results of the biological and thiochrome methods [3] was good in the fortified samples. At the lower concentrations of the original juice and wines the thiochrome readings were irregular and unreliable and are not quoted.

[3] The thiochrome determinations were made by Elsie O. Weast.

Riboflavin: Twelve-ounce bottles of clear or black glass were used to store portions of each of the Burger juices and wines in the laboratory in order to determine the rate of loss of riboflavin under the influence of light and at room temperature. Preliminary assays were made within a month of bottling the samples and again after 10 months. At the same time similar samples kept in the refrigerator at 40 to 50°F., and thus only occasionally exposed to light, were also examined for riboflavin.

The effect of the light was striking and consistent (Table 2). All juices and wines, whether reinforced or not, lost a large part of the ribo-

TABLE 2

Riboflavin Content of Burger Grape Juices and Wines as Affected by SO_2, Addition of Vitamins, and Conditions of Storage

Sample	Storage period	Temperature	Stored in light or dark	Riboflavin		Added riboflavin retained
				By rat-growth method	By microbiological method	
	mo.	*°F.*		*μg./100 gm.*	*μg./100 gm.*	*pct.*
Juice A	1	65	In dark	....	40	....
	10	65	In dark	48	16	....
	10	45	Partly in dark	25	16	....
	10	65	In light	53	10	....
Juice B	1	65	In dark	....	145	105
	10	65	In dark	95	140	124
	10	45	Partly in dark	110	135	119
	10	65	In light	30	22	12
Wine 2988A	1	65	In dark	....	25	....
	10	65	In dark	25	18	....
	10	65	In light	25	0	....
Wine 2988B	1	65	In dark	....	98	73
	10	65	In dark	98	87	69
	10	45	Partly in dark	75	85	68
	10	65	In light	30	35	35
Wine 2989A	1	65	In dark	....	27	....
	10	65	In light	11	15	....
Wine 2989B	1	65	In dark	....	107	80
	10	65	In dark	120	100	73
	10	45	Partly in dark	....	122	95
	10	65	In light	70	29	14
Wine 2990A	1	65	In dark	....	22	....
	10	65	In dark	19	14	....
	10	65	In light	0	5	....
Wine 2990B	1	65	In dark	....	104	82
	10	65	In dark	120	110	96
	10	45	Partly in dark	93	105	91
	10	65	In light	40	10	5
Wine 2991A	1	65	In dark	....	33	....
	10	65	In dark	33	5	....
	10	65	In light	10	0	....
Wine 2991B	1	65	In dark	....	112	79
	10	65	In dark	....	91	86
	10	45	Partly in dark	75	75	70
	10	65	In light	25	6	6

flavin when exposed to light in clear glass bottles. Those bottled in dark glass retained a large part of the vitamin during the 10 months' storage. Some of the reinforced samples which were kept at a lower temperature and only partially protected from light showed somewhat greater loss of riboflavin than did the dark bottled samples even though the latter were stored at higher temperatures.

TABLE 3

Pyridoxine and Pantothenic Acid Contents of Grape Juices and Wines

Sample	Storage at 60-70°F.	Pantothenic acid by microbiological assay	Recovery of added pantothenic acid	Pyridoxine by bioassay	Recovery of added pyridoxine
	mo.	*μg./100 gm.*	*pct.*	*μg./100 gm.*	*pct.*
Burger Juice A	6	150	.....	182	.....
Burger Juice B	6	1050	90	280	98
Burger Wine 2988A	6	110	.....	.....	.....
	18	.....	.....	70	.....
Burger Wine 2988B	6	1100	99	.....	.....
	18	.....	.....	200	130
Burger Wine 2989A	6	75	.....	.....	.....
Burger Wine 2989B	6	1050	97	120	.....
	18	.....	.....	120	.....
Burger Wine 2990A	6	65	.....	.....	.....
Burger Wine 2990B	6	1000	93	166	.....
Burger Wine 2991A	6	40	.....	.....	.....
	18	.....	.....	66	.....
Burger Wine 2991B	6	700	66	180	.....
	18	.....	.....	170	104
Tokay Juice 411	6	89	.....	83	.....
Tokay Juice 412	6	288	100	183	100
Tokay Wine 411a	6	92	.....	72	.....
Tokay Wine 412a	6	257	84	214	142

There seemed to be no clear relationship between the riboflavin retention and SO_2 content although Wine 2988B, which had the greatest amount of free SO_2, had slightly lower riboflavin values than the other reinforced wines.

The agreement between the results obtained by biological and microbiological methods[4] was good in most cases, particularly in the samples containing the larger concentrations of riboflavin. Both methods appeared unreliable when applied to samples containing less than 30 micrograms of the vitamin per 100 grams. The differences between the reinforced wines and juices stored in the light and in the dark were significant in every

[4] Some of the microbiological determinations were made by Marion B. Smith and Relda Cailleau.

case. There was no evidence of increase in riboflavin with fermentation as suggested by Flavier (1939) although an earlier experiment supported the suggestion, Morgan *et al.* (1939).

Pyridoxine: There seemed to be a larger amount of pyridoxine in all the juices and wines than was the case with riboflavin and thiamin. The added pyridoxine also seemed to be stable in the bottled samples over a period of 18 months at room temperature of 60 to 70°F. The unfortified juices and wines contained 66 to 182 micrograms of pyridoxine per 100 grams, and the fortified samples 120 to 282, indicating good retention of the added vitamin (Table 3). Only one of the fortified samples, Wine 2988B, showed appreciable loss of the added pyridoxine. Neither SO_2 treatment nor storage affected the stability of this vitamin.

The method of Scudi (1941) for the colorimetric determination of pyridoxine was used on these samples but with discouraging results. The recovery of added pyridoxine was unsatisfactory and the presence of other phenols interfered with the color readings. The values obtained after repeated trials were usually several-fold greater than those found by the rat bio-assay. For example, the Tokay juices and wines appeared to contain from 570 to 690 micrograms of pyridoxine per 100 grams by this method and 72 to 214 by the bio-assay; the Burger juices and wines appeared to contain 170 to 410 by the chemical method and 66 to 280 by the bio-assay. Preliminary extraction with ether and extraction with water-saturated butanol removed some of the interfering phenols but their

TABLE 4

Vitamin Content of Aged Commercial Wines

Type of wine	History	Riboflavin	Pantothenic acid
		µg./100 gm.	*µg./100 gm.*
Chablis	Aged 5 to 6 years	21	35
Sauterne	Semillon grapes, aged 5 yr.	....	40
Champagne	.....	....	25
Riesling	Aged in oak casks 5 yr., bottled 1 yr.	....	25
Tipo White	.....	12	25
Tipo Red	.....	12	35
Zinfandel	Zinfandel grapes, aged 4 yr., 12% alcohol	22	45
Angelica	Sultana grapes, aged 2 yr.	10	20
Muscatel	Muscat grapes, aged in redwood 4 yr., pasteurized, and filtered	6	15
Sherry	Palomino, Feher Zago, and Mission grapes, baked 5 mo. at 138°F., aged 3 to 5 yr.	17	7
Port	Zinfandel grapes, aged 7 yr. in wood, 19% alcohol	12	15

concentration appeared to be too great in these wines to allow complete removal of all non-pyridoxine compounds reacting with the chloroimide reagent.

Pantothenic Acid: The added pantothenic acid was well retained by both juices and wines (Table 3). There appeared to be relatively little loss of this vitamin owing to fermentation, the greatest loss occurring in Wine 2991 which was fermented without sulfite treatment. Unchecked bacterial growth in these samples may have caused the destruction of about 35 per cent of the pantothenic acid of the juice. In any case there was no adverse effect of SO_2 on the retention of this vitamin.

Commercial Wine Samples: Eleven samples of wines [5] of known history were procured from California vintners and examined by the microbiological method for riboflavin and pantothenic acid (Table 4). The range of riboflavin values, six to 22 micrograms per 100 grams, is lower than that of the experimental wines (Table 2), possibly because the former were aged two to seven years and nearly all were bottled in clear glass. The pantothenic acid contents, seven to 45 micrograms per 100 grams, also were lower than those found in the experimental wines (Table 3). The fortified (with distillate) wines—sherry, port, muscatel, and angelica—had less pantothenic acid than the dry wines but about the same range of riboflavin values.

SUMMARY

Two lots of grape juices and wines made from them were reinforced with different amounts of thiamin, riboflavin, calcium pantothenate, pyridoxine, and nicotinic acid and examined for the various vitamins immediately and after varying periods and conditions of storage.

Thiamin was found to be lost during vinification in the unreinforced wines in proportion to the amount of free SO_2 retained. In all but one wine, which retained 69 per cent, 85 to 100 per cent of the added thiamin was present after three months' storage at 7.2°C.(45°F.). The thiochrome method applied to these samples yielded results concordant with those of bio-assay only in the vitamin-fortified samples.

Riboflavin disappeared in both juice and wines exposed to light in clear bottles but was largely retained, 68 to 96 per cent, in the wines bottled in dark glass. Protection against light was more effective than moderately lowered temperature in conserving riboflavin. Rat-growth and microbiological methods of assay for this vitamin agreed fairly well, particularly in the analysis of the vitamin-reinforced samples.

Pyridoxine was stable in all samples over a period of 18 months as determined by biological assay. The chemical (Scudi) method for pyridoxine was not applicable to these samples because of the concentration of other phenols which reacted with the chloroimide reagent.

Pantothenic acid was well retained in all samples except that which had not received sulfite treatment.

Eleven samples of aged commercial wines bottled in clear glass were found to have lower riboflavin and pantothenic acid contents than the experimental wines.

[5] The samples were procured through L. D. Adams and the California Wine Institute.

CONCLUSION

Grape juices and wines retain the added vitamins, thiamin, riboflavin, pyridoxine, and pantothenic acid well in storage, except that riboflavin, natural and added, is largely destroyed in the juices and wines bottled in clear glass.

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ADDENDUM

In 1944, when the Burger juices and wines had been aged for four years at room temperature, the riboflavin determination by the microbiological method was repeated. All samples bottled in clear glass had only 1 to 8 micrograms riboflavin per gram. The vitamin-fortified wines 2988B, 2989B, 2990B, and 2991B (no SO_2) which were stored in black glass bottles had, respectively, 100, 78, 30, and 6 per cent of their original riboflavin content. The unreinforced wines 2989A and 2990A, bottled in black glass, retained 33 and 36 per cent of their riboflavins. The samples which had received no SO_2 treatment after fermentation suffered the greatest loss of the vitamin.

EFFECTS OF METHODS OF BLANCHING, STORAGE, AND COOKING ON CALCIUM, PHOSPHORUS, AND ASCORBIC ACID CONTENTS OF DEHYDRATED GREEN BEANS [1]

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Although there has been a great impetus in dehydration and in research on dehydration, there still remains considerable disagreement concerning the nutritive value of dehydrated vegetables. Chace (1942) stated that if care were taken, dehydrated foods would carry more vitamins to the consumer than the average fresh product obtained from the food market. Farrell and Fellers (1942) found, however, that the ascorbic acid content of green beans was almost completely destroyed by dehydration. Mathiesen (1939) and Moyer (1943) have found large losses of ascorbic acid in the dehydration of vegetables. Cruess and Mrak (1942) stated that the relative vitamin content of fresh and dehydrated vegetables has not as yet been fully determined.

There is considerable disagreement on the best method of blanching vegetables for dehydration. The water blanch, especially the serial water blanch, is commonly used in England. In serial water blanching the water is used over and over again, a process which has been found to reduce losses of soluble nutrients. In this country steam blanching is preferred since it is thought that soluble nutrient losses can be reduced by steam blanching. Kanitz and Dammann (1940), German workers, found that blanching beans in water which contained $NaHSO_4$ and Na_2SO_4 increased the retention of their nutritive value, particularly of ascorbic acid. Beckley and Notley (1941) stated that unblanched beans contained more ascorbic acid after dehydration than blanched beans. They recommended that beans be dried without blanching.

The present work was undertaken to study the effect of the following treatments on the calcium, phosphorus, and ascorbic acid contents of green beans: five methods of blanching; one method of dehydration; and four methods of storage of the dehydrated beans for six months. Another experiment was conducted to study the effect of two methods of cooking on the calcium, phosphorus, and ascorbic acid contents of dehydrated green beans.

EXPERIMENTAL PROCEDURE

Statistical Design of Experiment:[2] A factorial design of experiment was planned for four lots of green beans. Landreth's Stringless Green Pod

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[2] The statistical design of experiment was planned by Dr. Boyd Harshbarger, Agricultural Economics Department, Virginia Agricultural Experiment Station, and the analysis of the data was carried out under his supervision.

bush beans were planted on different dates so that they would mature at four different times during the season. They were grown by the Department of Horticulture under similar soil and fertilizer conditions, harvested, and brought to the laboratory early on the date of harvest. They were washed, the ends were removed, but the beans were left whole. The lot was mixed well and 20 samples were taken. Four of these samples were blanched by each of five methods and then dehydrated. After dehydration these samples were stored for six months by four different methods. Thus a total of 80 samples of green beans was studied for nutritive value after blanching by five methods, after dehydration, and after storage for six months by four methods. The analysis of variance was used to determine significant differences among means.

A separate experiment was set up to test the difference in the conservation of nutrients when dehydrated green beans were cooked without rehydration and when they were cooked after rehydration. Twelve lots were cooked by each of the two methods and the nutritive value of the cooked lots was compared with that of the dehydrated beans. Analysis of variance was again used to study the data.

Blanching Methods: The five blanching methods used were as follows: (1) blanched 18 minutes in tap water; (2) blanched 18 minutes in tap water to which had been added $NaHSO_4$; (3) blanched 18 minutes in tap water to which had been added $NaHSO_4$ and Na_2SO_4; (4) blanched 30 minutes in a wire rack in steam; and (5) not blanched, to be used for check lots. The time of blanching was counted from the instant the vegetable was placed in the boiling water or the steam. The length of time of blanching had been determined previously as the time necessary for peroxidase to become completely inactivated as indicated by the benzidine test, Proctor (1942). These times of blanching were sufficiently long so that the beans could be considered precooked. Lids were used on the kettles for all five blanching methods. The amounts of $NaHSO_4$ and Na_2SO_4 added were sufficient to lower the temporary hardness of the tap water from 200 to 105 parts per million (expressed as $CaCO_3$). Kanitz and Dammann (1940) found a higher retention of ascorbic acid in beans that had been blanched in water in which the temporary hardness had been partially converted to permanent hardness by addition of the sulfates.

Dehydration Process: The 20 samples from each lot were dehydrated in a dehydrator[3] designed for the experiment so that each tray, which contained a different sample, would receive the same air flow and heat treatment as all the other trays. This experimental dehydrator was very satisfactory since the drying rate for all the trays was practically the same. In a preliminary experiment equal weights of blanched beans were placed on the trays. After the drying period it was found that the weights of the beans dehydrated on the different trays varied only from 50.4 to 51.8 grams, a variation of 2.7 per cent. To rule out possible unrecognized variations, the trays containing the various samples were randomized for

[3] Designed and operated by Mr. P. B. Potter, Associate Agricultural Engineer, Virginia Agricultural Experiment Station.

position in the dehydrator. The beans were dried on glass trays in circulating air at an air temperature which was held between 65.6 and 71.1°C. (150 and 160°F.) for most of the drying period. The beans were dried until brittle. The length of drying time was nine hours for the first lot and 10 hours for the second, third, and fourth lots. The dehydration process usually ended about midnight. The beans were left in the dehydrator until morning when the samples were put into paper bags and stored 24 hours in a closed tin can. This ripening period was used to cause uniform distribution of moisture throughout the sample.

Storage Methods: As explained above, the four samples from each blanching method for each lot were stored after dehydration by four methods for six months. These methods were as follows: (1) in fruit jars in a refrigerator; (2) in cellophane bags in a refrigerator; (3) in fruit jars at room temperature; and (4) in cellophane bags at room temperature. Rubbers and zinc lids were used on the fruit jars. The bags were made from No. 450 MST cellophane and were enclosed in cardboard cartons. The temperature of the refrigerator used for storage ranged from 0 to 10.5°C. (32 to 51°F.) and the relative humidity from 31 to 75 per cent; the average temperature was 6.1°C. (43°F.) and the average humidity was 55 per cent. The temperature of the room in which the samples were stored ranged from 20 to 30.5°C. (68 to 87°F.) and the relative humidity from 10 to 56 per cent; the average temperature was 23.3°C. (74°F.) and the average humidity was 34 per cent.

Cooking Experiment: An amount of beans sufficient for the cooking experiment was picked from the first planting. They were steam blanched for 30 minutes and dehydrated until brittle. The dehydrated beans were mixed well and 12 samples of 40 grams each were treated by each of the following methods: (1) 800 ml. of tap water was brought to boiling and the sample of dehydrated beans was dropped into the water and cooked for 30 minutes; (2) 800 ml. of cold tap water was used to soak the dehydrated beans overnight (15 to 17 hours). The water was then drained off, brought to boiling, and the rehydrated beans were added and cooked for 30 minutes. Three grams of salt was added to each cooking lot. The lids were left off during cooking. The cooked beans, the cooking water, and the dehydrated beans were analyzed for ascorbic acid. The cooked and dehydrated beans were analyzed for calcium and phosphorus.

Methods of Analyses: All beans (except those for the cooking experiment) were analyzed after blanching for dry matter, calcium, phosphorus, and ascorbic acid. Ascorbic acid determinations were again made after dehydration and after storage.

For the determination of dry matter, samples were dried to remove most of the moisture, ground, and dried in circulating air at 72°C. (161.6°F.). The methods for calcium and phosphorus determinations were the official methods of the Association of Official Agricultural Chemists (1940).

Ascorbic acid was determined on the first two lots by the Heinze *et al.* (1944) modification of the Morell (1941) method, using one per cent HPO_3 as the extractant. However, as the season advanced the seeds in the beans became larger and evidently an increased protein content interfered with the use of this method. Therefore, it was necessary to use the

original Morell (1941) method for the last two lots. In the latter method a three-per cent HPO_3 solution was used which prevented the interference of protein. After extraction of the sample was made, the extract was adjusted to a pH of 3.6, an aliquot was added to the indophenol dye, and the sample was read in an Evelyn photolometer.

RESULTS AND DISCUSSION

All data were analyzed by the analysis of variance. If a significant difference among means was found at any level, i.e., at the five-, one- or 0.5-per cent level, the value for the difference necessary for significance between any two of the means was found, using the five-per cent level.

Values for loss of dry matter during blanching and the calcium and phosphorus contents of the blanched beans (Table 1) show that the date

TABLE 1

Effect of Date of Harvest and Method of Blanching Green Beans on Their Dry-Matter Losses and Calcium and Phosphorus Contents

Harvest and blanching	Dry matter lost during blanching (raw)	Calcium content of blanched beans (dry basis)	Phosphorus content of blanched beans (dry basis)
	gm./900 gm.	*pct.*	*pct.*
Date of harvest:[1]			
7/12/43	11.47	.56	.57
7/26/43	7.48	.32	.36
8/24/43	9.74	.45	.38
8/30/43	10.32	.39	.40
Difference necessary between means for significance at 5% level	2.36	.05	.02
Method of blanching:[2]			
Tap water	7.87	.46	.43
Tap water + $NaHSO_4$	11.46	.45	.43
Tap water + $NaHSO_4$ + Na_2SO_4	11.11	.47	.43
Steam	8.57	.41	.43
Raw	.....	.37	.42
Difference necessary between means for significance at 5% level	2.36	.05	....[3]

[1] These values are means of 20 samples. [2] These values are means of 16 samples. [3] No significant differences among means.

of harvest had a significant effect, since the least loss of dry matter—7.48 grams per blanched lot of 900 grams of raw beans—was found in the second harvest made July 26. This loss was significantly less than those for the first and last harvests, made July 12 and August 30, respectively. The method of blanching also caused significant differences in loss of dry matter. The blanching water containing $NaHSO_4$ and that containing $NaHSO_4$ and Na_2SO_4 caused significantly more loss of dry matter than steam and tap-water blanching. The 900 grams of beans blanched in water containing $NaHSO_4$ lost 11.46 grams and those blanched in water containing $NaHSO_4$ and Na_2SO_4 lost 11.11 grams, while those blanched in tap water and in steam lost, respectively, 7.87 and 8.57 grams.

The date of harvest caused significant differences in the calcium content among all four lots of green beans. Beans harvested July 12 had the high-

est content, 0.56 per cent dry basis, while those harvested July 26 had the lowest, 0.32 per cent. The phosphorus content also varied significantly for each date of harvest. Beans harvested July 12 had the highest phosphorus content, 0.57 per cent dry basis, while those harvested July 26 had the lowest content, 0.36 per cent.

The method of blanching affected the calcium content of the blanched beans. Calcium content of green beans blanched in water, whether tap water or water to which $NaHSO_4$ or $NaHSO_4$ and Na_2SO_4 had been added contained significantly more calcium, 0.46, 0.45, and 0.47 per cent dry basis, respectively, than the raw beans which contained 0.37 per cent calcium. Those blanched in steam contained 0.41 per cent calcium, dry basis, which was not significantly different from the raw beans. There were no significant differences in the phosphorus content of raw beans and beans blanched by any of the four methods. The phosphorus content varied only from 0.42 to 0.43 per cent dry basis.

The significant differences in dry-matter losses, and the calcium and phosphorus contents of green beans owing to date of harvest are likely due to climatic conditions. For the year and place these harvests were made, beans planted and harvested early in the season were richer in minerals than those harvested later. In regard to blanching, it is obvious that beans blanched in tap water containing 200 parts per million temporary hardness (as $CaCO_3$) and in tap water containing sulfates (temporary hardness reduced to 105 parts per million) take up calcium from the water and contain more than the raw beans. The phosphorus content is not changed during the blanching of whole green beans. It is obvious that these conclusions for minerals, made from analysis of beans after blanching, would apply as well to the beans after dehydration and storage since there can be no further change in calcium and phosphorus contents.

The effects of date of harvest, method of blanching, and method of storage on the ascorbic acid content of green beans after blanching, after dehydration, and after storage are given (Table 2); date of harvest was again a significant factor in the composition of green beans. The blanched beans were found to contain significantly less ascorbic acid with each advancing date of harvest. Beans harvested July 12 contained 2.49 mg. of ascorbic acid per gram dry basis, while those harvested at the end of the season, August 30, contained only 1.28 mg. The beans after dehydration still showed differences in ascorbic acid content owing to date of harvest. The last harvest, August 30, was significantly lower in this vitamin, 0.13 mg. per gram dry basis, than all the other harvests, which ranged from 0.25 to 0.30 mg. per gram. Those harvested August 24 were also lower than those harvested July 12. After storage of the dehydrated beans for six months, differences owing to date of harvest were no longer significant and the values ranged from 0.11 to 0.19 mg. per gram.

The method of blanching did not appear to have much effect on retention of ascorbic acid in the dehydration of green beans. Beans blanched by the four methods, i.e., tap water, tap water plus $NaHSO_4$, tap water plus $NaHSO_4$ plus Na_2SO_4, and steam, did not differ significantly in their ascorbic acid content (Table 2) after blanching, since the ascorbic acid content varied only from 1.64 to 1.73 mg. per gram dry basis. The raw

beans, of course, contained significantly more ascorbic acid, 2.33 mg. per gram dry basis, than any of the blanched beans. After dehydration there were still no significant differences among blanched beans, which ranged in ascorbic acid content from 0.21 to 0.27 mg. per gram dry basis. Unblanched or raw beans lost significantly more of their ascorbic acid than blanched beans, for they contained only 0.01 mg. per gram dry basis after dehydration. After storage for six months the blanched beans varied in ascorbic acid content from 0.13 to 0.18 mg. per gram dry basis, values which were not significantly different. Again the unblanched beans were much lower than the blanched, since they contained 0.03 mg. of ascorbic acid per gram dry basis.

Method of storage caused significant differences in retention of ascorbic acid during six months of storage. Dehydrated beans stored in the refrigerator in cellophane bags or in fruit jars contained significantly more ascorbic acid, 0.19 and 0.21 mg. per gram dry basis, respectively, than beans stored in cellophane bags at room temperature, which contained only 0.09 mg. Beans stored in fruit jars at room temperature were also significantly lower in ascorbic acid, 0.13 mg. per gram dry basis, than those stored in fruit jars in the refrigerator.

Thus, date of harvest seemed to be the most significant factor in the ascorbic acid content of dehydrated green beans since beans harvested early in the season were highest in this factor. The blanching method

TABLE 2

Effect of Date of Harvest, Method of Blanching, and Method of Storage on Ascorbic Acid Content of Green Beans After Blanching, Dehydration, and Storage [1]

Factors studied	Ascorbic acid content [2]		
	After blanching	After dehydration	After storage
	mg./gm.	*mg./gm.*	*mg./gm.*
Date of harvest:			
7/12/43	2.49	.30	.17
7/26/43	1.98	.27	.16
8/24/43	1.52	.25	.19
8/30/43	1.28	.13	.11
Difference necessary for significance at 5% level	0.20	.04	.... [3]
Method of blanching:			
Tap water	1.69	.27	.13
Tap water + $NaHSO_4$	1.64	.24	.15
Tap water + $NaHSO_4$ + Na_2SO_4	1.69	.24	.18
Steam	1.73	.21	.17
Raw	2.33	.01 [4]	.03 [4]
Difference necessary for significance at 5% level	0.23	.... [3]	.... [3]
Method of storage:			
Fruit jars in refrigerator	.....	....	.21
Cellophane bags in refrigerator	.....	....	.19
Fruit jars in room	.....	....	.13
Cellophane bags in room	.....	....	.09
Difference necessary for significance at 5% level	.....	....	.08
Average for blanched beans	1.69	.24	.16

[1] Figures calculated on dry basis. [2] All values given are means of 16 samples except those for date of harvest, after blanching, which are means of 20 samples. [3] No significant differences among means for blanched beans. [4] The difference between these values and those for blanched beans were obviously significant.

made no difference in ascorbic acid content of dehydrated beans. After dehydration, beans retained ascorbic acid best if stored in fruit jars in a cold place. Mean values of ascorbic acid for raw, blanched, dehydrated, and stored, dehydrated beans were 2.33, 1.69, 0.24, and 0.16 mg. per gram dry basis (Table 2). This represented a loss from the original amount in the raw beans of 27.5 per cent by blanching, 62.2 per cent by dehydration, and 3.4 per cent by storage. All these losses were statistically significant. Thus, a total of 93.1 per cent of the original ascorbic acid content was lost or there remained in the dehydrated stored beans only 6.9 per cent of the amount of ascorbic acid that was present in the raw beans. These results compared well with those of Farrell and Fellers (1942), who found that 95 per cent of the original ascorbic acid in green beans was destroyed in dehydration. The losses of this vitamin by the dehydration process are obviously very large.

Cooking Experiment: The results of losses owing to the two cooking methods (Table 3) show that the calcium content of the cooked, dehydrated

TABLE 3

Comparison of Dehydrated (Uncooked) Green Beans With Those Rehydrated and Cooked, and Not Rehydrated and Cooked for Calcium, Phosphorus, and Ascorbic Acid Contents, Weights After Cooking, and Losses of Ascorbic Acid Owing to Oxidation and Solution

Factor	Green beans[1]			
	Dehydrated	Not rehydrated, cooked	Rehydrated, cooked	Difference necessary for significance at 5% level
Calcium, pct. dry basis	.31	.39	.42	.03
Phosphorus, pct. dry basis	.43	.39	.36	.01
Ascorbic acid, mg. per gm. dry basis	.33	.10	.03	.04
Weight of vegetable in each lot, gm.	40.0	168.8	184.7	6.4
Distribution of ascorbic acid in the cooked lots:				
Ascorbic acid in the vegetable, mg. per lot	12.21	2.94	.25	.....
Ascorbic acid in the cooking water, mg. per lot	.....	7.23	3.31	1.33
Ascorbic acid oxidized, mg. per lot	.....	2.04	8.65	1.14
Total	.....	12.21	12.21	.....

[1] All values given are means of 12 samples.

beans was significantly higher than that of the dehydrated beans, and the beans rehydrated before cooking were still higher than those cooked directly. The dehydrated beans contained 0.31 per cent calcium, on the dry basis, while those cooked without rehydration contained 0.39 and those rehydrated before cooking, 0.42 per cent.

Cooking dehydrated beans caused significant losses of phosphorus. The beans rehydrated prior to cooking lost significantly more phosphorus than those cooked directly in boiling water. The values for the phosphorus content of dehydrated green beans, of beans cooked after rehydration, and of beans not rehydrated and cooked were 0.43, 0.36, and 0.39 per cent dry basis, respectively.

The ascorbic acid content of the cooked, dehydrated beans varied in the same way as phosphorus; that is, the content of the cooked beans was

significantly lower than the dehydrated beans and the beans which were rehydrated prior to cooking were significantly lower than those cooked directly. The ascorbic acid content of dehydrated green beans, of beans not rehydrated and cooked, and of beans rehydrated and cooked were 0.33, 0.10, and 0.03 mg. per gram dry basis, respectively.

The distribution of losses of ascorbic acid during cooking (Table 3) indicates that out of a total of 12.21 mg. of ascorbic acid in the lot, 2.94 was retained in the vegetable cooked without rehydration, 7.23 mg. was in the cooking water, and 2.04 mg. was oxidized. Only 0.25 mg. was found in the beans cooked after rehydration, 3.31 mg. was in the cooking water, and 8.65 mg. was oxidized. These values, calculated on the percentage basis, give the following results: for beans cooked without rehydration 24 per cent of the ascorbic acid was retained by the vegetable, 59 per cent was dissolved in the cooking water, and 17 per cent was oxidized; for beans cooked after rehydration two per cent of the ascorbic acid content was retained in the cooked beans, 27 per cent was dissolved, and 71 per cent was oxidized.

The weight of the lot of 40 grams of dehydrated beans after cooking was 168.8 grams for those not rehydrated and 184.7 for those rehydrated. The difference was significant.

In summary, dehydrated beans were found to be higher in calcium after cooking than before. This increase in calcium content was greater in beans which were rehydrated before cooking than in those cooked directly. The dehydrated beans lost significant amounts of phosphorus and ascorbic acid during cooking. However, losses of these nutrients were higher in beans that had been rehydrated prior to cooking than in those cooked directly. Beans absorbed more water when rehydrated than when cooked directly but it was thought that the average person could not detect the difference in plumpness and tenderness.

SUMMARY AND CONCLUSIONS

Date of harvest was a very important factor in the nutritive value of dehydrated green beans. Beans harvested early, July 12, 1943, were much higher in calcium, phosphorus, and ascorbic acid content than those harvested at the end of the season, August 30.

Although method of blanching made no significant difference in the ascorbic acid and phosphorus contents of dehydrated green beans it did make a difference in the calcium content of the beans. Beans blanched in tap water or in tap water to which had been added $NaHSO_4$ or $NaHSO_4$ and Na_2SO_4 contained more calcium than the raw beans. Steam-blanched beans were not significantly different from the raw beans in calcium content. Unblanched beans were definitely lower in ascorbic acid content than blanched beans after dehydration.

Method of storage affected the ascorbic acid content of dehydrated green beans stored six months. The best retention of ascorbic acid was in fruit jars in a refrigerator while the poorest retention was in cellophane bags stored at room temperature.

Rehydration of dehydrated green beans before cooking increased losses of phosphorus and ascorbic acid over beans cooked without rehydration.

Both methods of cooking, however, gave significant losses of phosphorus and ascorbic acid. The calcium content of beans cooked after rehydration was significantly higher than that of the beans cooked directly with no soaking. The calcium content of all cooked dehydrated beans was higher than the uncooked.

In general, dehydrated green beans were found to be as high in phosphorus as raw beans and higher in calcium. However, dehydrated green beans are a poor source of ascorbic acid, since only 6.9 per cent of the amount in the raw beans remained in the blanched beans after dehydration and storage for six months.

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A SIMPLE FLUOROMETRIC METHOD FOR THIAMIN IN MILK

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The method to be described is a simplification of the method reported by Hennessey and Cerecedo (1939). The modification was introduced since the original method did not clearly outline a method of extraction for milk products, and because the use of the base-exchange procedure with milk products proved to be difficult and no extraction solvent or mixture was found which yielded an extract easily adopted to the base-exchange step. The extraction method finally adopted is extremely simple, appears quantitative, and together with a correction step in the fluorometry affords a quick and reasonably accurate method for the determination of thiamin in milk and milk products. The reagents used in the method are as follows:

1. Quinine sulfate — fluorescent standard, concentrated solution. Dissolve 0.0108 gram of quinine sulfate in sufficient tenth normal sulfuric acid to make a liter of solution. Store in the dark in an amber-colored bottle. The working standard is prepared by diluting 2.5 ml. of the concentrated solution to 100 ml. with tenth normal sulfuric acid. The fluorescence of this solution is approximately equal to that given by the thiochrome from one microgram of thiamin in 13 ml. of isobutyl alcohol.

2. Thiamin hydrochloride—100 milligrams per liter of 20 per cent alcohol at pH 3.5-4.5. Store in an amber-colored bottle in a refrigerator. Dilute 1 to 100 with water for a working standard. This contains one microgram per ml. Discard any solutions showing mold growths.

3. Sodium hydroxide—15 grams dissolved in 85 ml. water.

4. Potassium ferricyanide solution—one gram per 100 ml. stored in an amber bottle in a cool, dark place.

5. Isobutyl alcohol with a low degree of fluorescence. Merck and Company's product has been found satisfactory.

6. Anhydrous sodium sulfate—reagent grade.

7. Taka-diastase—Parke Davis. This reagent should be analyzed by a blank determination on a water suspension and the thiamin content of the blank subtracted from that of the unknown if necessary.

8. Two per cent trichloroacetic acid solution—two grams in 98 ml. of water.

SPECIAL EQUIPMENT

1. Three centrifuge reaction vessels, 30 ml. content, 3¾ x 1$^{13}/_{16}$ inches (E. Machlett & Sons, N. Y. and also Pfaltz and Bauer, Inc., N. Y.).

2. Centrifuge—hand or power driven which can be adapted to use with the special reaction tubes.

3. Fluorometer.

4. Oven, incubator, water or oil bath that can be maintained at 50°C.

EXPERIMENTAL PROCEDURE

Pipette 10 ml. of the milk into a 50-ml. volumetric flask, add 50 mg. of taka-diastase, adjust the pH to 4.0 to 4.5 and incubate for three hours at 50°C. One should shake the flask occasionally to break up the curd that forms. After three hours the flask is cooled and diluted to the mark

with two per cent trichloroacetic acid, thoroughly mixed, allowed to come to equilibrium by standing for a few minutes, and filtered through a fairly coarse paper. The filtrate need not be absolutely clear, but if very cloudy it may often be further clarified by passing it through the filter again.

A five-ml. aliquot of the filtrate is pipetted into each of the three centrifuge reaction vessels marked 1, 2, and 3. To reaction vessel No. 2 are added one ml. of a solution containing exactly one microgram of thiamin chloride. To vessels No. 1 and No. 2 are added 0.1 ml. of the ferricyanide solution followed immediately by three ml. of the sodium hydroxide solution. Thirteen ml. of isobutyl alcohol are added to the mixtures in the three vessels, and finally three ml. of sodium hydroxide solution are added to the mixture in Vessel 3. The glass stoppers are then placed in the vessels and they are vigorously shaken for 1.5 minutes. This is followed by centrifuging for about one minute to separate the aqueous and isobutyl alcohol layers. The aqueous layer is drained off and discarded and the isobutyl layer is dried with a small amount of anhydrous sodium sulfate. The order of adding the reagents or the timing of the steps should not be changed. If the isobutyl alcohol layer does not separate completely, this may often be improved by swirling the mixture gently and recentrifuging. It may also be advantageous to recentrifuge after the addition of the sodium sulfate.

The fluorophotometer should have been adjusted so that it is operating properly according to the directions supplied by the manufacturer before the chemical reactions are begun and it should always be allowed to warm up 10 or 15 minutes before use. The dilute quinine sulfate solution is placed in one of the cuvettes and the diaphragm of the fluorophotometer adjusted so that this gives a half-scale deflection when placed in the instrument. The exact point chosen is not critical but the same reference point should be used for the three readings on any one sample. A reading B is taken on the thiochrome solution from Vessel 3 (the blank), a reading (X) is taken on the thiochrome solution from Vessel 1, and a reading (X + 1) is taken on the solution from Vessel 2. The calculation is simple:

$$\frac{X-B}{(X+1)-X}\ (1.0) = \text{micrograms of thiamin per ml. of milk or milligrams per liter of milk.}$$

The fluorophotometer readings may be used directly for the calculation if the instrument gives a linear relationship between thiamin concentration and the galvanometer readings. If it does not do this, it is necessary to convert the readings to thiamin by use of a standard curve before substitution can be made in a formula similar to that given above.

For milk powders a suitable sample is weighed into a volumetric flask reconstituted with water and is then treated identically with the fresh or evaporated milk. Evaporated milk is not diluted for the thiamin determination.

RECOVERY DETERMINATIONS

Recovery experiments were made by adding thiamin chloride to samples of fresh and evaporated milk; the results of these experiments are tabu-

lated (Table 1). The recovery range is somewhat wider than might be desired. Hennessey and Cerecedo (1939) report recoveries of 90 to 97 per cent for their enzymatic thiochrome method. This indicates less variability but consistently low recoveries.

TABLE 1

Recovery of Thiamin Added to Milk

Sample No.	Thiamin per liter				
	In sample	Added	Found	Recovered	Recovered
	EVAPORATED MILK				
	mg.	*mg.*	*mg.*	*mg.*	*pct.*
1	.52	1.00	1.51	0.99	99
2	.27	1.00	1.34	1.07	107
3	.48	1.00	1.50	1.02	102
4	.38	1.00	1.32	0.94	94
5	.43	1.00	1.43	1.00	100
Average	....	.....	.....	.....	100
	FRESH MILK				
1	.44	1.00	1.46	1.02	102
2	.26	1.00	1.30	1.04	104
3	.36	1.00	1.41	1.05	105
4	.27	1.00	1.22	0.95	95
5	.20	1.00	1.20	1.00	100
Average	....	.....	.....	.....	101

The use of taka-diastase to liberate thiamin from cocarboxylase as suggested by Hennessey has been included in the method, although it is not entirely clear whether the value of this preparation lies chiefly in its dephosphorylating properties or its proteolytic activity. Other investigators have suggested the need for papain or pepsin to free thiamin from thiamin-protein combinations. Since preparations of the Parke-Davis taka-diastase used in this work demonstrated milk clotting and other proteolytic enzymes, we have not found it necessary to use other enzyme preparations. As is demonstrated (Table 2) low values are consistently obtained on samples of fresh milk when taka-diastase is omitted. The difference for evaporated milk is smaller and of doubtful significance, but it is probably wise to include the step in routine analyses.

R. R. Williams and his colleagues, in their work on the molecular structure of thiamin, made considerable use of the reaction of sodium bisulfite in splitting thiamin into the pyrimidine and thiazole halves. The use of this reagent to prepare vitamin-free diets for assay work has been suggested; and Kline, Hall, and Morgan (1941) have shown that the reaction is quantitative at pH 3.2 to 6.0. The specificity of the analytical method reported here has been checked with bisulfite treatment at pH 4.0 to 4.5. Five samples of fresh milk, five samples of evaporated milk, and two samples of dry skim milk powder were analyzed after bisulfite treatment. None of the samples contained any apparent thiamin and this fact gives considerable evidence that the method is specific for thiamin.

The results secured with the fluorometric method have been compared with those secured by microbiological methods. For this work the method

TABLE 2

Thiamin Content of Milk With and Without Use of Taka-diastase

Sample No.	Thiamin per liter	
	Without taka-diastase	With taka-diastase
Evaporated Milk (Undiluted)		
	mg.	*mg.*
1	.40	.45
2	.39	.40
3	.49	.50
4	.35	.37
5	.38	.41
6	.37	.38
7	.43	.42
Average	.40	.42
Fresh Milk		
1	.27	.26
2	.20	.33
3	.19	.39
4	.19	.32
5	.19	.24
6	.20	.42
7	.21	.27
Average	.21	.32

TABLE 3

Comparison of Results Secured With Fluorometric and Microbiological Methods for Thiamin

Sample No.	Thiamin content per liter	
	Fluorometric method	*Phycomyces blakesleeanus* method
Fresh Milk		
	mg.	*mg.*
1	0.49	0.57
2	0.39	0.38
3	0.47	0.57
Dry Skim Milk		
Sample No.	Thiamin content per kilogram	
	Fluorometric method	*Phycomyces blakesleeanus* method
	mg.	*mg.*
1	4.7	4.2
2	4.6	4.4
3	4.8	4.5
Evaporated Milk (Undiluted)		
Sample No.	Thiamin content per liter	
	Fluorometric method	*S. salivarius* S20B method
	mg.	*mg.*
1	0.56	0.55
2	0.55	0.55
3	0.55	0.52
4	0.58	0.52

of Hamner, Stewart, and Matrone (1943) which uses the mold, *Phycomyces blakesleeanus,* was chosen. The data (Table 3) show there is good agreement in the results secured by the two methods when they are applied to fresh and dry skim milk. The data presented are similar to those secured by Hamner, Stewart, and Matrone (1943) in their comparison of the mold method and the Hennessey and Cerecedo (1939) thiochrome method as applied to plant materials.

The *Phycomyces* method consistently gave higher results than the fluorometric method when applied to evaporated milk. This is believed to be caused by the fact that *Phycomyces blakesleeanus* responds to the pyrimidine and thiazole cleavage products of thiamin together as well as to thiamin. Data secured in our laboratory indicate that a part of the thiamin destruction in the sterilization of evaporated milk results in the formation of cleavage products which are biologically active for *Phycomyces blakesleeanus.*

The microbiological method of Niven and Smiley (1943) has been used to compare with the fluorometric results on evaporated milk, since this method uses the organism, *S. salivarius* S20B, which does not respond to the thiazole or pyrimidine cleavage products singly or together. The agreement shown (Table 3) supports our belief that the high results secured on evaporated milk by the *Phycomyces blakesleeanus* method are due to the thiamin cleavage products. Knott (1942) has previously shown that the pyrimidine product is present after the sterilization and storage of evaporated milk and has suggested the destruction is the result of a simple cleavage.

The fluorometric and *S. salivarius* methods have not been compared as to the results given on fresh or dry skim milk. Since Niven and Smiley (1943) obtained good agreement when the latter method and the *Phycomyces blakesleeanus* method were applied to fresh and dry skim milk, and since the data in Table 3 show good agreement between the *Phycomyces blakesleeanus* and fluorometric methods, further work seemed superfluous.

SUMMARY

A simple fluorometric method for the determination of thiamin in milk has been presented. By recovery experiments and by sulfite treatment of representative samples, it is shown that the method is reasonably accurate and specific for thiamin.

Results secured by the fluorometric method agree with those secured by the *Phycomyces blakesleeanus* method when the two methods are applied to fresh or dry milk. Good agreement is likewise shown between the fluorometric and *S. salivarius* methods when they are applied to evaporated milk. High results on evaporated milk secured by the *Phycomyces blakesleeanus* method support the belief that the thiamin destruction which occurs in the sterilization of evaporated milk is in part at least a simple cleavage into the pyrimidine and thiazole components of thiamin.

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RETENTION OF ASCORBIC ACID IN MARMALADE DURING PREPARATION AND STORAGE[1]

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Many fruits used in the preparation of marmalades, jams, and preserves are high in ascorbic acid—namely, citrus fruits, black and red currants, strawberries, and raspberries. From the standpoint of food economy and public health, the ascorbic acid content of these fruits should not be wasted when they are converted into marmalades and jams. It is important to retain this ascorbic acid even though we recognize that jams and marmalades contain only 45 per cent fruit and are ordinarily consumed in comparatively small amounts. Tasty fruit spreads are always in demand. They can contribute important nutrients to the diet and encourage people to eat more bread, both of which are especially important during war years. Marmalades and jams have proved so popular with the men in our armed forces that the Army and Navy are interested in nutritious fruit spreads.

Citrus marmalade is prepared using both the juice and peel of citrus fruits. Citrus juice has long been known as an antiscorbutic agent but not until recently has the high ascorbic acid content of peel been generally recognized. As early as 1754, however, Lind reported the case of a man who, by eating the discarded skins of lemons, did not develop scurvy. Later, Hess and Fish (1914) found that an orange-peel infusion prevented scurvy in infants. Citrus peel is from two to five times higher in ascorbic acid than is the juice. Marmalade is one form in which citrus peel has been used acceptably as a human food.

Bacharach, Cook, and Smith (1934) report that Delf in 1921 made a biological estimation of the vitamin C in marmalade. Delf found that the orange marmalade which she prepared contained about one-tenth of the antiscorbutic value of fresh orange juice. Delf assumed that the vitamin C in marmalade was derived entirely from the orange juice used. Later, Bacharach, Cook, and Smith (1934) found that the larger the amount of peel contained in marmalade, the greater the ascorbic acid content. Cheftel, Panouse, Hoag, and Boisse (1941) found, as did Bacharach, Cook, and Smith (1934), that peel did contribute to the ascorbic acid content of marmalade. Sedky, Fellers, and Esselen (1942) produced a high vitamin C marmalade but did not attribute any of the ascorbic acid content to the peel. Bacharach, Cook, and Smith (1934) found that commercial marmalades contained little ascorbic acid. The range of values which they report are from six to 14 mg. per 100 grams. Sedky, Fellers, and Esselen (1942)

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found a range of 2.1 to 6.3 mg. ascorbic acid per 100 gm. of commercial marmalade. None of these workers have reported studies of the ascorbic acid content of citrus marmalade during storage.

The need for further study of the ascorbic acid content of citrus marmalade is indicated. The problem seems to require, first of all, a comparative study of the different methods of vitamin C assay; second, confirmation of the reports that ascorbic acid is present in commercial marmalade in only insignificant amounts; and third, an investigation of the preparation and storage of citrus marmalade to determine if the ascorbic acid *can* be retained during the preparation and cooking of the fruit and during the storage of the final product.

EXPERIMENTAL PROCEDURE

The determination of reduced and total ascorbic acid was made by titration with 2,6-dichlorophenolindophenol, by the photoelectric indophenol method and by the method of Roe and Kuether (1943) and Roe and Oesterling (1944).

In obtaining the samples, care was taken to avoid any contamination with copper and iron. For juice, five-gram samples were used; for orange peel, 20-gram samples; for lemon peel, 10-gram samples; and for marmalade, at first 20-gram and later 30-gram samples were used. The peel was cut into thin strips and well mixed before the sample was taken. When sampling the marmalade there was no difference noted in the ascorbic acid content of the top layer and the lower layers. This has likewise been noted by Hoffman La Roche laboratories (1941).

The samples of juice were made to a known volume with three per cent metaphosphoric acid or five per cent metaphosphoric in 10 per cent acetic acid, depending upon the assay method to be used. The samples of peel and marmalade were extracted five to seven minutes in a Waring blendor with the acid medium, centrifuged, and the supernatant liquid made to a known volume with the acid medium. One extraction was found to be sufficient for the marmalades and two for most of the peels. It was found that reproducible results could be obtained more easily by extracting the marmalade samples in a known amount of the acid, filtering, discarding the first portion of the filtrate, and using an aliquot of the remaining filtrate. The dilution effect of the moisture in the fresh sample was considered in the calculation.

The extracted samples were stored overnight at 3.3°C.(38°F.). Samples stored at 38°F. showed no decline in ascorbic acid content for a period of 48 hours, but metaphosphoric acid extracts left at room temperature for longer than 24 hours lost an appreciable amount. Mapson and Mawson (1943) obtained similar results.

In order to determine whether or not light has any effect on metaphosphoric extracts of citrus peel, duplicate samples were allowed to stand in strong daylight and in the dark at room temperature. There was no appreciable difference in ascorbic acid content of the samples at the end of three days.

To determine total ascorbic acid, H_2S was slowly bubbled for 15 minutes through a portion of the extract; the extract was stoppered and

allowed to stand for 15 minutes. The excess H_2S was removed with a stream of carbon dioxide. The dye solution was standardized against an ascorbic acid solution of known concentration. A faint pink coloration which persists for 15 seconds was the end point to which the titrations were made. When using the photoelectric colorimeter, two ml. of dye were pipetted into a colorimeter tube, two ml. of extract buffered to pH 3.6 were quickly blown from a pipette into the dye, the mixture was shaken, and the tube placed in the photoelectric colorimeter within five seconds after the addition of the extract to the dye. Readings of the galvanometer were made at 15, 30, and 45 seconds. The turbidity was corrected for in the calculations. The phenylhydrazine method was followed as described by Roe and associates (1943), (1944). Because the standard curves obtained were not reproducible, it was necessary to run standards with each group of samples.

The titration values were on the average slightly higher than were the values obtained using the photoelectric colorimeter. This is to be expected. The coefficient of correlation between the penylhydrazine method and titration after reduction with H_2S was 0.9053. Thus the variation among the samples by one method accounts for 90 per cent of the variation among the samples by the other method.

COMMERCIAL MARMALADES

Several commercial varieties of citrus marmalade were purchased from local stores. The ascorbic acid content of these marmalades was determined. The average range of values found was from 4.4 to 9.1 mg. per 100 gm. Since the date of manufacture and the history of storage of these commercial marmalades were not known, we had no way of ascertaining whether these marmalades had been high in ascorbic acid when first prepared and had lost ascorbic acid content during storage or whether the marmalades were manufactured in such a way as to destroy the ascorbic acid present in the fruit used. A freshly prepared sample of commercial marmalade was obtained, however, and was found to contain 10 mg. of ascorbic acid per 100 gm. Upon subsequent storage for four months at 37.8°C.(100°F.) this sample gave a value of 7.4 mg. per 100 gm. These values are in line with those reported by other workers and noted earlier in this paper, thus indicating that the procedure employed in the commercial manufacture of citrus marmalade is destructive of ascorbic acid.

PREPARATION OF HIGH VITAMIN C MARMALADE

After many trials, using various proportions of ingredients, a marmalade of high quality was developed which was also high in vitamin C. This marmalade formed the basis of further studies. The proportion of ingredients and the directions for making it are given below.

Citrus Marmalade

2 quarts water	1,000 grams sugar
150 grams orange peel	800 grams orange juice
50 grams lemon peel	200 grams lemon juice

Method: Add the water to the thinly sliced peel; cook slowly in a large covered pan until the peel is tender. Add the sugar to the peel and peel liquid. Boil the mixture rapidly until it begins to give the jelly test. Add the fruit juice and continue cooking until the mixture just gives the jelly test (about 220°F.). Pour the marmalade into clean, hot glasses and seal.

About 60 ml. of cider vinegar per liter were added to the water in which the peel cooked. This was done because citric acid was not available at the time and it was thought that vinegar would help effect a lower pH. It has been shown by Hoffman La Roche laboratories (1941), however, that the stabilizing effect which citric acid has on ascorbic acid is not a matter entirely of pH.

The average ascorbic acid value obtained for orange juice was 51 mg. per 100 gm., the range being from 44 to 68 mg. per 100 gm. For lemon juice we obtained an average value of 47 mg. per 100 gm. and a range of 33 to 54 mg. per 100 gm. The orange peel which we used gave, on the average, 115 mg. ascorbic acid per 100 gm. and a range of 94 to 184 mg. per 100 gm. Lemon peel averaged 178 mg. ascorbic acid per 100 gm. and the range of values was from 139 to 223 mg. per 100 gm. These values agree well with values which have been published by many workers.

It is known that an appreciable amount of the ascorbic acid content of fresh fruit may be destroyed in the preparations required before cooking. By cutting orange peel into thin strips a loss of only seven per cent ascorbic acid was found when the peel was allowed to stand at room temperature for 30 minutes. This loss was accompanied by an increase of seven per cent dehydroascorbic acid. The approximate time required for cutting all of the peel in preparation for cooking was 30 minutes. Grated orange peel lost 43 per cent of its ascorbic acid content and increased in dehydroascorbic acid content 16 per cent by standing at room temperature for 30 minutes. Ground orange peel lost 12 per cent ascorbic acid and increased in dehydroascorbic acid by 10 per cent. Lampitt and Baker (1942) made a study of the disappearance of ascorbic acid in minced orange peel exposed to air. Their studies showed a loss of 75 per cent after three hours. The ascorbic acid of orange and lemon juice is fairly stable to oxidation. There is little loss when the juice remains uncovered in the room for one or two hours.

In seven batches of this marmalade there was an average ascorbic acid content after cooking of 28.6 mg. ascorbic acid per 100 gm. and an average retention in preparation of 68 per cent. The range of values in the cooked marmalade was from 22 to 34 mg. per 100 gm.; the range in per cent retention was from 46 to 85. There was no difference noted in the ascorbic acid content of the marmalade or the per cent ascorbic acid retention when the peel was cooked in a three-per cent sugar solution, in deaerated water, in deaerated distilled water, or in 0.2-per cent citric acid solution. Further study is needed in order to determine the precautions of Cheftel *et al.* (1941), which are truly worth while.

It may be noted that in the above formula the ratio of fruit to sugar is 1.2 : 1; in commercial formulae it is more likely to be 0.5 : 1. The higher ratio of fruit helps produce a marmalade with distinctive natural fruit flavor and color, good consistency and keeping qualities, and a greater potentiality for a high ascorbic acid content. This same formula can be used with twice as much sugar to produce marmalade which is good in all qualities including ascorbic acid content but it in no way comes up to the high standards of the marmalade described previously.

Marmalade made with 100 gm. of orange peel, 75 gm. grapefruit peel, 25 gm. lemon peel, 425 gm. orange juice, 200 gm. grapefruit juice, 55 gm. lemon juice, and 700 gm. sugar gives an excellent product containing from 45 to 50 mg. ascorbic acid per 100 gm. The larger amount of ascorbic acid in this marmalade is undoubtedly due to the grapefruit peel, since it is reported by DeWitt and Sure (1938) to contain about 265 mg. per 100 gm.

It is possible to make a marmalade containing 25 mg. of ascorbic acid per 100 gm. from peel. Cheftel *et al.* (1941) made peel marmalade by cooking one kg. of shredded peel in about five liters of 1.6 per cent citric acid and three per cent sugar solution. When the peel was tender, 2.5 kg. sugar were added and the mixture cooked to a definite final weight. The work of Cheftel *et al.* (1941) was confirmed in that a peel marmalade was prepared containing 28 mg. ascorbic acid per 100 gm. by following the same general directions and proportions. A peel marmalade, containing 40 mg. ascorbic acid per 100 gm. was also prepared using grapefruit and orange peel. When grapefruit peel is used, not as much citric acid is needed. It would seem that grapefruit peel should be included in high vitamin C marmalades.

STORAGE OF MARMALADE

In order to study the effect of storage temperature on the retention of ascorbic acid in citrus marmalade, sealed samples from six batches of freshly prepared high vitamin C marmalade were stored at 37.8, 30, and 3.3°C.(100, 86, and 38°F.), respectively. The samples were assayed for

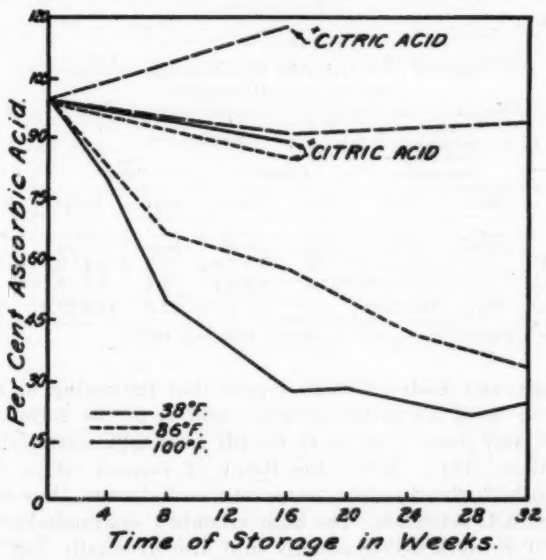

FIG. 1. Retention of ascorbic acid in citrus marmalade under different storage temperatures.

Because of the rather great variability among samples of the same marmalade, the values for this figure are averages of all the like marmalades tested within a definite period of storage time. The marmalades which were stored for only 16 weeks were prepared by cooking the peel in 0.2-per cent citric acid solution.

ascorbic acid at varying intervals from one to 260 days; the results obtained are given schematically (Fig. 1).

The variability of ascorbic acid content between samples of the same marmalade is great. It is believed that this is due largely to the difference in the amount and kind of peel contained in the sample. It is clearly evident from Fig. 1 that there is a rapid disappearance of ascorbic acid in samples stored at 100° F., less at 86°F., and at 38°F. the amount remains more or less constant. There is also an indication that citric acid helps stabilize the ascorbic acid in marmalade during storage.

Wokes, Organ, and Jacoby (1943) report that foods containing carbohydrate and pectin may be particularly susceptible to the formation of compounds simulating vitamin C and not distinguishable from it by the usual indophenol method when stored at high temperatures. Wokes *et al.* (1943) term these compounds "apparent vitamin C." The "true ascorbic acid" values of samples from four batches of stored marmalade were determined, using the method described by Mapson (1943); the results are given (Table 1). In the samples of marmalade which were prepared and stored in such a way that they retained large amounts of ascorbic acid, the "apparent vitamin C" value represents only five per cent of the total. When a marmalade has been prepared and stored so that it contains very little ascorbic acid, the percentage becomes much greater. Also the amount of nonspecific dye-reducing substances seems to be greatest in the marmalades stored at 100°F. The values (Fig. 1) were not corrected to "true ascorbic acid" values.

TABLE 1

Determination of Nonspecific Dye-Reducing Substances in Stored Citrus Marmalade [1]

Treatment	Total indophenol reducing substances		Ascorbic acid				"True ascorbic acid"	
			"Apparent"		"True"			
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
	mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*pct.*
Stored at 100°F..	3.3	2.8- 4.3	2.2	1.9-2.9	1.2	0.4- 2.4	34	12-56
Stored at 86°F....	5.1	3.0- 9.3	1.8	1.6-2.1	3.4	1.3- 7.2	59	43-77
Stored at 38°F....	23.4	16.8-29.1	1.2	1.2-1.2	22.2	15.6-27.9	95	93-96

[1] Each value represents the average of samples from four batches.

Hamburger and Joslyn (1941) report that darkening of citrus juice does not occur until all of the ascorbic acid is in the form of dehydroascorbic acid and there is no more readily oxidizable material present in the juice. Ross (1943) found the flavor of canned citrus juice to be dependent on both the time and temperature of storage, thus roughly paralleling vitamin C retention. The high vitamin C marmalades which were stored at 100°F. darkened gradually and also gradually lost in ascorbic acid content. The marmalade made with citric acid did not decrease in ascorbic acid to as marked a degree as did the others but it did darken. The dehydroascorbic acid content was rather constant and not dependent upon the darkening of the marmalades. The darkening did not seem to affect the flavor of the marmalade to any marked degree.

SUMMARY AND CONCLUSIONS

The determination of reduced and total ascorbic acid in citrus juice, peel, and marmalade was made by titration with 2,6-dichlorophenolindophenol, by the photoelectric indophenol method and by the phenylhydrazine method.

The ascorbic acid content of the commercial marmalades sampled ranged between four and 10 mg. ascorbic acid per 100 grams.

A citrus marmalade was prepared containing an average of 28.6 mg. ascorbic acid per 100 gm. with an average ascorbic acid retention during preparation of 68 per cent. There is a rapid disappearance of ascorbic acid in marmalades stored at 100°F., less rapid at 86°F., and practically no loss at 38°F. Cooking the peel in 0.2-per cent citric acid solution seems favorable to the retention of ascorbic acid during storage.

The nonspecific dye-reducing substances in stored citrus marmalade may be considered as insignificant when large amounts of ascorbic acid are present.

Marmalade containing between 25 and 30 mg. of ascorbic acid per 100 gm. should be of significant value as an antiscorbutic agent. Marmalade can easily be prepared containing this amount. By making high vitamin-C marmalade with citric acid and storing it at cool temperatures, it is possible to have good retention of the vitamin.

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STABILITY OF THIAMIN CHLORIDE (VITAMIN B_1) IN MINERAL AND VITAMIN-FORTIFIED CEREAL

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Various problems have arisen in connection with the fortification of cereals. Whether the reasons are of lasting importance is doubtful, but industry has found psychological reasons for attempting to replace the lost food factors without changing the physical character of refined cereals. This requires, as a rule, addition of pure vitamins and mineral salts to the cereal, and then using practical means for shortening the cooking time so that the added vitamins will not be destroyed by the processing of the product in the home.

Because of the general interest in these newly fortified products, it seemed timely to examine those commonly used in the home in order to learn whether enrichment had brought about the desired result in the product after it had been "quick-cooked" and was ready to place on the table. In a study of the effect of long cooking of cereals by Hanning (1941) it had been determined that while cooking caused little loss of the thiamin contents of natural wheat and oat cereals, the vitamin-supplemented ones showed a loss in thiamin content as determined by the rat-growth method.

In selecting the cereal for study, it seemed important to determine which of the newer commercial products would be most likely to show a loss of thiamin during the cooking process. It had been reported by Williams and Spies (1938) that the destructibility of thiamin by heat was higher in alkaline than in acid media. Moreover, Copping and Roscoe (1937) had reported that no loss of thiamin occurred during the baking of yeast bread, the pH of which was 5.5 to 5.9, whereas there was considerable loss during the baking of baking-powder bread (pH 6.9 to 7.2). It seemed reasonable to expect, therefore, that if the quick-cooking process were capable of causing destruction of thiamin added to cereals during the enrichment process, such destruction would be especially evident in the cereal having the highest pH during the cooking process. Accordingly, we made our selection of the cereal for investigation on the basis of degree of alkalinity.

EXPERIMENTAL PROCEDURE

Twelve commercial brands of cereals were tested for their pH values in order to select one for the assay work. Some of these were in natural form while others were fortified with various minerals or vitamins or both. The cereals were prepared according to the directions on the containers,

[1] The data in this paper are based upon the thesis presented by Alice B. Eklund in partial fulfillment of the requirements for the degree of Master of Science in Home Economics at the University of California at Los Angeles, February, 1943.

the quick-cooking method being followed when a choice was indicated. The pH values of both raw and cooked cereals were determined, using the Beckman pH Meter.

The descriptions of the cereals tested and the pH's found for them are indicated (Table 1). The cereal which was highest in pH value was selected for investigation. It obviously owed its tendency toward alkalinity to the presence of small, added amounts of tricalcium and disodium phosphates.

TABLE 1

pH Values of Natural and Fortified Cereals in the Raw and Cooked States

Description of cereal	Type of enrichment	Vitamin unitage as per label	pH value—raw	pH value—cooked
1. Wheat cereal, fine, white.....	None (natural form)	.....	5.39	5.48
2. Wheat cereal, fine, white.....	Vitamin D	.....	5.42	5.51
3. Wheat cereal, fine, white.....	Wheat germ and salts of iron, calcium, and phosphorus	.....	5.10	5.55
4. Wheat cereal, fine, white.....	Thiamin chloride	900 I.U. B_1 per pound	5.33	5.55
5. Wheat cereal, darker.....	Wheat germ	.....	5.45	6.08
6. Wheat cereal	Whole wheat, wheat germ	61.2 I.U. B_1 per ounce	5.95	5.98
7. Rolled oats	None (natural)	.....	5.95	5.95
8. Rolled oats, quick.....	None (natural)	.....	5.90	6.05
9. Wheat cereal	Wheat germ, yeast, mineral salts	.....	5.98	.....[1]
10. Wheat cereal, entire (100%)	None (natural)	.....	6.05	6.10
11. Wheat cereal	Wheat germ, irradiated yeast	3600 I.U. B_1 per 2 pounds	6.37	6.28
12. Wheat cereal, fine, white.....	Thiamin chloride, wheat germ, tricalcium phosphate, disodium phosphate, iron	50 I.U. B_1 per ounce	7.10 7.50	6.90 7.26

[1] This cereal was of the "prepared" or already cooked type, not requiring cooking at home.

The rat-growth method of thiamin assay as described by Booher and Hartzler (1939) was used. This method is based upon a comparison of the average gains in weight induced in suitably standardized young rats receiving known and unknown amounts of B_1 added to a thiamin-free basal ration. Thus the foodstuff with unknown B_1 content could be evaluated. Such comparisons were made strictly within each litter, using paired littermate feeding technique to compare the growth induced by a given weight of cooked with the same weight of raw material. When the size of the litter permitted, there were two pairs of rats on each of two levels of

cereal, one animal of each pair being on raw and one on cooked cereal. There were usually two positive control rats (one of each sex) each receiving a daily dose of one International Unit (three micrograms) of thiamin chloride. For a group of animals consisting of three or four litters there were two to four negative controls, receiving no thiamin supplement.

Three groups of animals were used, 85 in all. Immediately after weaning, at the age of 21 to 25 days and within a weight range of 35 to 45 grams, the rats were individually caged in false-bottomed assay cages of standard mesh and were placed upon a thiamin-free basal diet until they were depleted of their storage of the vitamin as evidenced by cessation of growth. This required 14 to 19 days.

Following the thiamin-depletion period, the rats were given daily supplements of thiamin (for the positive controls) and of the cooked and raw cereal (for the experimental animals) during the three-weeks test period. In order to save time, the supplements were fed in carefully weighed, double portions every second day. In all cases, however, the supplements were accepted eagerly and were consumed quickly.

The thiamin-free basal diet was a modification of the one recommended by Kline, Tolle, and Nelson (1938),[2] which was kept in small, tightly sealed black jars refrigerated until needed. The protection from light was a precaution used to retain the riboflavin of the yeast in the basal diet. This basal diet was fed *ad libitum* except for the short periods during which the supplements were being consumed.

The cereal supplements were from a freshly opened package of the fine-grained, white, enriched cereal which had been selected. One half of the package was quick-cooked as follows:

To six parts of boiling water were added a teaspoon of salt and one part by measure of cereal, and the mixture was cooked over lowered heat for just five minutes after the boiling temperature had been reached.

The cooked cereal was cooled, its pH value was determined, and it was dried at a temperature of not over 50°C.(122°F.). It was next ground to a fine consistency, similar to that of the uncooked cereal, and was stored in the refrigerator. Since the cooked cereal was dried until it attained the approximate weight of the original product, the animals were fed

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[2]

	Per cent		Per cent
Sucrose	60	Salt mixture	4
Sulfite-treated yeast	10	Fat (corn oil)	6
B_1 free casein	18	Sardine oil	2

The yeast used was brewer's blended yeast residuals (Anheuser Busch, Los Angeles). It was freed of thiamin specifically by sulfite treatment as recommended by Kline, Tolle, and Nelson (1938).

The casein (Golden State Creamery, Los Angeles) had been rendered approximately free of vitamins of the B-complex group by washing with fresh water 11 times during the process of manufacture. In order to insure complete destruction of thiamin, the sulfite-treated yeast was poured over the casein and the mixture was dried at a temperature of about 50°C.(122°F.).

The salt mixture was the modified Osborne and Mendel one, suggested by Booher and Hartzler (1939).

The sardine oil (Van Camp Sea Food Co., Inc., Terminal Island, California) was a preparation with added vitamin A and contained 3,000 International Units of vitamin A and 400 of vitamin D per gram.

equal weights of the cooked and uncooked cereals when these were used as vitamin-containing supplements.

The positive-control rats were fed a supplement prepared from pure, crystalline thiamin chloride,[3] the solutions of which were prepared and preserved in accordance with the instructions of the American Pharmaceutical Association (1939). The final solution, made up at weekly intervals, was of such dilution that it contained the daily dosage of three micrograms in 0.1 ml.

RESULTS AND DISCUSSION

Upon termination of the three-weeks experimental period the average weekly gains of the rats were calculated. The statistical method described by Swift (1939) was used to determine which weights of each series should be eliminated[4] because of too great variation from the mean. Next the significance of the difference between average weekly gains induced by the raw and the cooked cereal was determined and was found to be negligible.[5] Finally, the thiamin contents of the raw and of the cooked cereal were calculated by comparison of growths induced by a known amount of thiamin chloride in comparison with those brought about by the cereal supplements.[6] The cooked cereal was favored (Table 2); it was found to contain 420 micrograms of B_1 per 100 grams, as against 320 micrograms for the same weight of raw cereal.

The somewhat better growth results of the animals on the cooked cereal point to the possibility that thiamin was more available to the rats when the cereal had gone through the cooking process. A similar result was

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[3] Winthrop Chemical Company, Inc.

[4] Only five animals were selected for elimination.

[5] The significance of the mean difference between average weekly growth rates of the rats on raw and on cooked cereal at each of the two levels was checked by the Student's "t" test used by Booher and Hartzler (1939). The following equation was used: $t = \frac{X\sqrt{N}}{S\,D}$, in which X represents the mean difference between pairs; N, the number of pairs; and S D, the standard deviation of a single measurement. For the rats on the higher level of cereal, for example, the mean difference between average weekly growth rates of the rats on the raw and on the cooked cereal was 1.9 grams with a standard deviation of 3.7. Calculation for "t" in the equation gave a result of 1.6, indicating a fairly low probability that the difference was significant. For the animals on the low level of cereal, the difference was even less likely to be significant, since "t" was found to be less than 1 (0.4).

[6] The values of the B_1 content of the raw and cooked cereals were determined by a method commonly used in biological assay, that indicated by Booher and Hartzler (1939). The average weekly weight increase of the positive control(s) in each litter was compared with those of animals receiving raw and cooked supplements of cereal. The amount of thiamin (X) in the daily supplement of cereal was calculated according to the equation: $A:X = B:C$, in which A was the amount of thiamin in micrograms fed to the control(s); B, the average weekly gain of the control(s); and C, the average weekly gain of the animal on cereal supplement. Where there were two control animals or two animals on the same weight of raw or cooked supplement, the two average weekly weight increments were averaged. The comparisons were first made within the litters. Later, the values received for X were converted to micrograms per 100 grams of cereal and were averaged to determine the amount of thiamin in the raw and in the cooked cereal, according to this assay.

pointed out by Kelly and Porter (1941) who found the thiamin more available in cooked than in raw beans.

It should be pointed out that while the conditions of the experiment allow one to conclude that there was no loss of thiamin upon the cooking of the cereal they do not permit any conclusion regarding a possible loss from the packaged cereal of some part of the vitamin added at the time of enrichment. In terms of International Units per ounce, the values received were 30 units for the raw cereal and 40 units for the cooked, levels somewhat lower than the value indicated on the package, which was approximately 50 International Units per ounce. The packages of cereal were of course not airtight and it was impossible to tell how long they had remained in the store after delivery from the producer. (Each package of cereal used for this experiment was purchased when needed, was opened at once, and half of it cooked immediately so that there would be a minimum loss of vitamin.) Moreover, as stated on the label, the thiamin value was an approximate one. It might be significant to mention, also, that the method of assay generally used by commercial companies is a chemical one, the results from which are not always comparable with those obtained by the rat-growth method of assay.

TABLE 2

Thiamin Content of Fortified Cereal Before and After Cooking

Cereal (finely ground, white)	Cooking time	Average pH values of cereal samples	Number of rats used in calculating results	Weight of cereal supplements	B_1 content of cereal
	min.			*gm./da.*	*μg./100 gm.*
Raw	....	6.79	30	0.8-1.0	320[1]
Cooked	5	6.99	32	0.8-1.0	420[1]

[1] These values were based on the results secured with the rats on the high level of cereal (0.8 to 1.0 gm. per day) since all of these animals had growth rates falling within the proper range. According to Booher, it appears that the rat-growth method of assay of vitamin B_1 as described here is sensitive to about 0.75 microgram of this vitamin when daily supplements are fed at levels which will induce average growth rates within the limits of eight to 14 grams per week. The majority of the animals on the low level of cereal (0.4 to 0.6 gm. per day) had average weekly growth rates of eight grams or less. Thus it was considered advisable not to use these figures in calculating the thiamin values for the raw and for the cooked cereal.

There can be no doubt of the efficacy of the basal diet used in this experimental work. That the sodium sulfite-treated yeast was thiamin free was demonstrated by each group of animals since they reached stationary weight in 15 to 19 days, and those kept on the unsupplemented diet, the negative controls, showed rapid losses in weight and eventually died of deficiency. All animals were at an optimum state of well-being at the beginning of the experiment. Moreover, upon addition of one International Unit of thiamin per day, growth commenced in all cases, thus bearing out the supposition that B_1 was the only factor of importance to the rat which was lacking in the basal diet.

It was evident from the results that the relatively high pH value of the cereal had little or no effect upon thiamin chloride stability during cooking. That the cereal was always at approximately the neutral point and that cooking caused no appreciable change in pH value is evident

(Table 3); the pH values of cereal samples used for the three groups of experimental animals are presented.

Studies made by other investigators on the effect of alkalinity of foods on the stability of thiamin do not lead to concordant conclusions. Certain of them would indicate significant losses of the vitamin when the medium was neutral or only slightly alkaline; others would indicate no such losses.

In the first group are found the reports of Fincke and Little (1941), Aughey and Daniel (1940), and Lantz (1938). Lantz did not report pH values but did find greater loss of thiamin from Pinto beans soaked in tap water or in water made slightly alkaline by addition of soda than occurred when the beans were soaked for 16 hours in distilled water. Fincke and Little (1941), working on baking powder and yeast bread, found losses of thiamin chloride when baking powder was used. However, in the case of muffins, no pH value was recorded and the cooking process itself did not seem to increase the loss of the vitamin. Aughey and Daniel (1940) had varying results. In the cooking of snap beans they found as great an increase in loss as 27 per cent in the beans cooked with soda over those cooked without soda, although the increase in pH was only from 5.8 to 6.6.

TABLE 3

pH Values of Raw and Cooked Cereal

Cereal sample	pH value—uncooked	pH value—cooked
I	7.18	6.98
II	6.80	6.79
III	6.93	7.20

The investigators reporting little or no loss of vitamin B_1 as a result of relatively alkaline media were Aughey and Daniel (1940) (results on the cooking of navy beans with and without soda); Kelly and Porter (1941); Barackman (1942); and Johnston, Schauer, Rapaport, and Deuel (1943). The study reported in the present paper agrees with the results of this group of authors.

Kelly and Porter (1941), investigating Michelite and Cranberry beans (Michigan), found that soaking for 16 hours in waters of varying alkalinity had no effect upon the B_1 content of the beans.

Barackman (1942) carried out a study of thiamin contents of self-rising bread and biscuits made from flour enriched by the addition of 2.16 milligrams of B_1 per pound. While he did observe poor recoveries of thiamin when sufficient baking powder was added to increase the pH of the mixture to 7.3 or higher, he reported optimum retention of thiamin when the pH did not exceed 7.1 to 7.2 during the baking process.

Johnston, Schauer, Rapaport, and Deuel (1943) reported even more striking resistance of food thiamin to destruction through alkalinity of cooking medium. Addition of soda to the cooking water, resulting in pH's ranging between 8 and 9, had no effect upon the loss of thiamin which occurred in peas during the cooking process. These workers pointed out, however, that thiamin losses owing to leaching out of materials into the cooking water are greatly increased by overcooking of the product.

The cooking time for fresh peas was reduced from 17 to eight minutes and for frozen peas, from six to four minutes by the addition of about one-quarter gram of soda to one cup of water.

It should not be concluded from the results reviewed here and from those reported in the present paper that thiamin might be regarded as stable under all cooking conditions in which the pH values of the media do not exceed 7.5 or 8. The destructive effect of high, dry heat, such as may be encountered in overbaking of biscuit, was pointed out by Barackman (1942); and, such as may be found at the surfaces of a loaf of bread in ordinary baking, was reported by Morgan and Frederick (1935).

Moreover, Beadle, Greenwood, and Kraybill (1943) pointed out the need for caution in relating stability of thiamin to variations in the pH of the food medium alone because of other factors playing an important part. There is evidence from their work that electrolyte systems are important in themselves. At the same pH (5.4) they found only slight destruction (three per cent) of pure thiamin in a phosphate medium, over 50 per cent loss in an unbuffered aqueous solution, and complete destruction in the presence of borates. In each type of solution, destruction would rise from 0 to 100 per cent within the range of 2 to 3 pH units. These authors agree with Melnick, Robinson, and Field (1941) in concluding that thiamin is more stable in biological tissues than in pure solution. It is quite possible that other factors, such as protein systems and the combinations of thiamin with phosphate, may also influence the stability of the vitamin.

It is evident therefore that with our present state of knowledge one cannot safely predict, in advance of experimental work, what may be the effect of a given heating process upon the thiamin content of a specific food product.

SUMMARY AND CONCLUSIONS

Eleven breakfast cereals, of the uncooked type, examined for their pH's in the raw and cooked states, showed a range of values from pH 5.1 to 7.5 in the raw state and from pH 5.5 to 7.3 when cooked.

The cereal which tested to give the highest pH value, a product fortified with thiamin chloride and minerals, was assayed for thiamin by the biological (rat-growth) method both before and after quick cooking to determine the effect of the cooking process upon thiamin stability.

The determined value for the raw cereal was 3.2 and for the cooked was 4.2 micrograms per gram.

It was concluded that quick cooking of this cereal caused no destruction of the added thiamin and possibly increased its availability to a slight extent.

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QUALITY OF EVISCERATED POULTRY OBTAINED FROM DEFROSTED, DRESSED STOCK

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The method of handling poultry as eviscerated stock holds many advantages. A primary advantage resides in the fact that taints of visceral origin are not imparted to edible tissues. It becomes obvious that if evisceration is delayed until after taints have been imparted to the meat, a main objective to be realized by employing the method has been defeated. It is only natural to expect that considerable time and effort should be expended by investigators in the industry in determining safe procedures to follow in handling eviscerated poultry.

Sair and Cook (1938) reported that the rate of freezing had little effect on the development of visceral taints and particularly emphasized that they developed rapidly after poultry was thawed. It is obvious that quality may be impaired by deteriorative changes if freezing is extremely slow. Cook (1939) emphasized that taint development appears to depend largely on the period during which stock is held above freezing temperature. Progressive deterioration may take place during the chilling, freezing, or thawing periods.

Fitzgerald and Nickerson (1939) found that chickens dressed and placed under suitable refrigeration within 70 minutes after killing could be held for a period of eight days without risk of off-flavor development. Their data would seem to indicate that in order to obtain these results, poultry must be held at temperatures very close to 0°C.(32°F.). Poultry held at temperatures close to 4.4°C.(40°F.) developed off-flavors within a period of two to three days.

Stewart, Lowe, and Morr (1941) reported the appearance of bile stains on livers within eight hours in dressed birds held at 1.7°C.(35°F.). Off-odors appeared in the kidney area after 24 hours and gradually spread throughout the bird reaching the wing joint in four days. A quite strong flavor was noticed in the thigh meat after stock was held four days at 1.7°C. Hanson, Stewart, and Lowe (1942) found that when dressed broilers were spray-cooled and stored at 1.7°C. decreases in aroma and flavor scores were obtained after 40 hours. The scores continued to decrease thereafter with an increase in the length of storage period prior to cooking. Stewart, Hanson, and Lowe (1943) froze birds by means of a brine spray within five hours after dressing and stored them up to six months before they were defrosted and eviscerated. The quality was superior to that of birds eviscerated 18 hours after dressing and stored for the same period of time.

A review of the literature indicates that there may be slight differences of opinion regarding the length of time and the temperatures required to impair quality when holding dressed poultry prior to the time that it is

eviscerated. In general, however, the results demonstrate that it is advantageous to eviscerate poultry promptly after it is dressed and if there is to be any delay in evisceration, it must be held near the freezing point of water or must be frozen promptly after it is dressed and chilled.

Further studies concerning the influence of time and temperature on quality of frozen-defrosted, dressed poultry, intended for later evisceration, are needed. The primary purpose of the present study was to determine the manner in which quality of poultry will be impaired by visceral taints if stock is not eviscerated immediately after dressed birds become defrosted.

EXPERIMENTAL PROCEDURE

A total of 60 fryer-size, battery-raised birds was used in this study. The birds were all handled similarly prior to being dressed. Each bird was individually given a "full crop" of feed and dressed approximately five hours later. The birds were semi-scalded and no wax was employed in the dressing operations. A freezing temperature of —23.3°C.(—10°F.) was employed. The eviscerated poultry was defrosted overnight at 10°C. (50°F.) before being cooked.

Three separate tests, which subsequently will be referred to as Tests A, B, and C, were conducted. Twenty birds were used in connection with each test and five methods of handling were employed for each test. There was considerable duplication with regard to methods of handling in the three tests in consequence of which only seven different methods of handling were employed in the experiment. The seven methods are as follows:

1. Warm-eviscerated, frozen—the birds were eviscerated immediately after dressing and promptly frozen.
2. Ice slush-chilled, frozen, defrosted, eviscerated, refrozen—these birds were dressed then ice slush-chilled three hours at 0.6°C.(33°F.) and then frozen. Later these birds were defrosted in running water and, as soon as thawed, were eviscerated and refrozen.
3. Air-chilled, frozen, defrosted, eviscerated, refrozen—birds were dressed and then chilled overnight at 2.2°C.(36°F.) and then frozen. When removed from the freezer, birds were defrosted in running water and were eviscerated and refrozen immediately upon becoming defrosted.
4. Air-chilled, frozen, defrosted, held 24 hours at 2.2°C., eviscerated, refrozen—poultry was dressed and chilled overnight at 2.2°C. prior to being placed in the freezer. Stock was defrosted in running water and held 24 hours at 2.2°C. before being eviscerated and refrozen.
5. Ice slush-chilled, frozen, defrosted, held 24 hours at 10°C., eviscerated, refrozen—poultry was ice slush-chilled three hours at 0.6°C. and frozen. Birds were defrosted in running water and held 24 hours at 10°C. before being eviscerated and refrozen.
6. Air-chilled, frozen, defrosted, held 24 hours at 10°C. in air, eviscerated, refrozen—dressed birds were chilled overnight at 2.2°C. before being frozen. They were defrosted in running water and then held 24 hours at 10°C. in air before being eviscerated and again frozen.
7. Air-chilled, frozen, defrosted, held 24 hours in water at 10°C., eviscerated, refrozen—poultry was chilled overnight at 2.2°C. before being frozen. Birds were defrosted in running water and then held 24 hours at 10°C. in water before being eviscerated.

The birds were cooked by roasting and a committee of three inspected them with regard to aroma and flavor. The aroma was scored as being

(5) very desirable, (4) desirable, (3) slightly desirable, (2) slightly undesirable, and (1) undesirable. The flavor of the dark and white meat was scored separately as being (5) excellent, (4) very good, (3) good, (2) fair, and (1) poor. At each inspection aroma and flavor scores were obtained on five birds, one bird being identified with each of the five methods of handling employed for a given test. Four such inspections were made of birds in case of each test.

EXPERIMENTAL RESULTS

The average aroma and flavor scores for Tests A, B, and C (Table 1) indicate that the warm evisceration of poultry followed by prompt chilling and freezing constitutes an excellent method of handling poultry as far as the aroma and flavor of the product are concerned. This method of handling poultry will serve as the control method against which all other methods of handling may be compared. The scores indicate that the poultry that was chilled rapidly, frozen, defrosted, and then eviscerated at once upon becoming defrosted and again frozen, also constituted an excellent product. Slightly less desirable but still a very acceptable and palatable product was poultry that had been air-chilled at 2.2°C. overnight prior to freezing and then eviscerated at once upon becoming defrosted.

The quality of poultry handled in accordance with the other methods had been impaired. By comparison, particularly with the warm-eviscerated poultry, this stock constituted inferior product. This included poultry that had been held at 2.2 or 10°C. for one day after being defrosted before it was eviscerated. The scores clearly demonstrate that if it does become necessary to hold defrosted poultry prior to evisceration, it is more desirable to hold it at 2.2°C. than at 10°C. There is some indication that rapid chilling followed by prompt freezing tends to delay the appearance of visceral taints in defrosted, dressed poultry.

It becomes evident that practices that delay evisceration, after dressed poultry has become defrosted, should be avoided.

DISCUSSION

There are many factors that may influence results obtained by investigators who depend upon aroma and flavor scores of product for quality evaluations. The time at which birds are inspected after cooking and the extent to which they are cooked are important. When stock is inspected at once after removal from the oven, the aroma is pronounced and may reflect the quality of the product more vividly than flavor. If there is a delay between removal of product from the oven and inspection, then flavor scores may reflect quality more vividly than aroma scores. The extent to which birds are cooked may influence flavor scores. Objectionable taints are more apparent in birds that are slightly undercooked and become more difficult to discern in the well-cooked birds. In the present test, poultry was well cooked and was examined promptly after removal from the oven.

The scores demonstrate that, organoleptically, warm evisceration constitutes an excellent method for handling eviscerated poultry. A considerable volume of poultry has been warm-drawn or warm-eviscerated during the past year. The method has received some criticism possibly because

TABLE 1

Aroma and Flavor Scores for Tests A, B, and C

Method of handling	Test A			Test B			Test C		
	General aroma	Dark meat flavor	White meat flavor	General aroma	Dark meat flavor	White meat flavor	General aroma	Dark meat flavor	White meat flavor
1. Warm-eviscerated, frozen	4.19	3.67	3.94	4.50	4.75	4.75	4.75	4.25	4.66
2. Ice slush-chilled, frozen, defrosted, eviscerated, refrozen	.....	.....	.....	4.37	4.75	4.62	4.33	4.33	4.25
3. Air-chilled, frozen, defrosted, eviscerated, refrozen	3.92	3.71	4.06	3.62	4.12	4.75	3.58	3.75	4.08
4. Air-chilled, frozen, defrosted, held 24 hours at 36°F., eviscerated, refrozen	3.56	3.52	3.87	.....	.....	.....	2.33	2.08	2.25
5. Ice slush-chilled, frozen, defrosted, held 24 hours at 50°F., eviscerated, refrozen	.....	.....	.....	2.87	3.75	4.50	.....	.....	.....
6. Air-chilled, frozen, defrosted, held 24 hours at 50°F. in air, eviscerated, refrozen	2.77	3.31	3.91	1.87	2.37	3.87	1.58	1.67	2.42
7. Air-chilled, frozen, defrosted, held 24 hours in water at 50°F., eviscerated, refrozen	2.62	3.24	3.64	.....	.....	.....	.....	.....	.....

it represents a distinct departure from previous practices employed by the poultry industry. There would appear to be no reason for criticizing the method on the basis of organoleptic considerations.

There has been a tendency in the industry to centralize evisceration operations. This development has been a logical one since certain economies are to be realized. Generally not enough local poultry is handled to permit an evisceration plant to continuously handle a large volume when relying exclusively on unfrozen poultry. In order to have sufficient volume available, dressed poultry has been frozen and then shipped to a central plant for evisceration. The aroma and flavor scores demonstrate that the method of rapidly chilling the product, followed by prompt freezing and prompt evisceration after defrosting, is entirely adequate from a quality viewpoint. In handling poultry in accordance with this method it is desirable to chill the stock rapidly and place it in a freezer on the same day it is dressed. Chilling the poultry in air at 2.2°C. for 24 hours and freezing it on the day following dressing is satisfactory although somewhat less desirable.

It was pointed out previously that the primary purpose of the present study was to determine the influence of time and temperature on the quality of frozen-defrosted-dressed poultry intended for subsequent evisceration. The results clearly indicate the manner and extent to which quality is impaired when dressed poultry is defrosted and not promptly eviscerated. In terms of visceral taints imparted to the edible tissues, quality is definitely lowered when poultry is held for 24 hours after it has become defrosted. This is evident even when the poultry is held at very adequate cooler temperatures, such as 2.2°C., and is particularly evident when it is held at a cooler temperature of 10°C. during the 24-hour period.

SUMMARY

Aroma and flavor scores demonstrate that the warm evisceration of poultry constitutes an excellent method for handling poultry in that visceral taints are not imparted to the edible tissues.

The quality of poultry is seriously impaired by visceral taints when defrosted-dressed poultry is held at 10°C.(50°F.) for one day or more prior to evisceration, and quality is impaired, although to a lesser degree, when the defrosted-dressed poultry is held at 2.2°C.(36°F.) for a period of one day or more.

Poultry intended for freezing and subsequent evisceration should be chilled rapidly and placed in an adequate freezer within 24 hours after dressing. It is preferable to freeze this poultry or place it in the freezer the same day it is dressed. Stock should be eviscerated at once after it has become defrosted.

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PROCESSING EQUIPMENT USED IN EMERGENCY MEAT DEHYDRATION RESEARCH BY UNITED STATES DEPARTMENT OF AGRICULTURE [1]

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Various general methods of processing, involving types of equipment and features of operation found successful with other products, were tested in the investigations. New equipment was designed and used. In consequence many combinations of equipment and conditions were studied during the course of the research on dehydration of meat.

PRECOOKING APPARATUS

The view was generally held that, preliminary to dehydration proper, it was necessary to subject meat to a heat treatment. This step in the processing performs various functions which will be explained at appropriate points in the series of papers.

There appeared to be several possible methods for accomplishing the precooking. One method was to utilize open, steam-jacketed kettles. Of those available, one type was made of steel and had a diameter of 27 inches with a depth of 19 inches. The other type was made of heavy cast iron and was 12 inches in diameter and 19 inches deep. It was jacketed only on the bottom in contrast with the other kettle, which was jacketed on both bottom and sides, but like the other was equipped with a drain for

[1] EDITOR'S NOTE. In response to war-emergency needs for meat in highly concentrated form that can be shipped without refrigeration, the Agricultural Research Administration of the United States Department of Agriculture, early in 1942, undertook research on the dehydration of beef, pork, lamb, and mutton. The work dealt primarily with dehydration processes but also included intensive studies of packaging, storage, chemical and bacteriological changes, compressibility, rehydration, nutritive value, utilization, and palatability of the products. The results have been published in somewhat condensed, semitechnical form in Circular 706 of the Department of Agriculture. The present paper is the first in a technical series planned for publication in *Food Research* which is designed to report on the different phases of the studies in all essential detail.

[2] When this work was done both Mr. Noel and Mr. Hollingshead were associated with the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

removing liquids at the bottom, a steam gauge, and a steam trap. The kettles were supplied with steam at a pressure from 0 to 65 pounds per square inch and the traps were equipped with a valve for collecting the condensate when its weight was required. The agitation of the meat in these kettles was by hand.

Another method of precooking was to place ground meat in any one of three dehydrators of vacuum-rotary type and apply heat with the stirring mechanism in operation. These machines are illustrated and described at later points in the article. In this method the processing is continued directly from precooking at atmospheric pressure to drying under vacuum without transferring the meat from one piece of equipment to another.

On the double-drum drier (Fig. 1) meat was precooked by dry heat. This machine could be heated to any required temperature by admitting steam into the hollow drums or rolls, each of which was three feet long and two feet in diameter. Steam pressure of 60 to 100 pounds was commonly used. A variable speed drive furnished the means for moving the drums at rates of 1.08 to 3 revolutions per minute. The space between the two drums could be varied from 0.09 up to 0.25 inch. Raw meat placed in the trough formed by the two drums adhered to one or the other of them through about three-fourths of a revolution, when it was scraped off by a stationary knife and fell into a pan.

Still another piece of precooking equipment used was a retort or autoclave (Fig. 2) made of steel plate, well trussed, with steel frame construction, having a large door on heavy steel hinges. It was approximately five feet long, three feet high, and three feet wide. Equipment consisted of a steam gauge, thermometer, safety valve, and steam trap for proper removal of condensate and any other waste liquids.

VACUUM DEHYDRATORS

Dehydration can be carried out at lower temperatures and the product can be reduced to a lower moisture content in vacuum than in air. However, generally speaking, vacuum drying is not so adaptable to large-scale operations as air-drying.

Extensive tests were conducted on vacuum-rotary Machine A (Fig. 3) which consisted of a steel shell with heavy cast-iron ends. A jacket covered all parts of the cylinder except the top and bottom openings and the ends. By a system of valves the temperature of the jacket could be controlled by admitting steam up to 30 pounds of pressure or water at any temperature desired. The over-all length of the cylinder was four feet and the diameter two feet. To a concentrically located shaft were attached four curved blades or paddles which served to agitate the product. The clearance between the paddles and shell was 0.25 inch. The speed of the shaft could be controlled at from three to 10 r.p.m. A port was provided on the top for filling the machine, the capacity of which was 300 pounds of ground, raw meat. There was a similar opening on the bottom for removing the finished product. The connection from the top of the dehydrator to the vacuum pump was a four-inch pipe. The temperature of the meat during the processing was determined by means of a thermometer, the bulb of which projected a short distance into the dehydration chamber through one of the end plates of the cylinder.

FIG. 1. Precooking meat on double-drum drier. Note the cooked meat that has been scraped off the drum and has fallen into the pan.

FIG. 2. Placing meat in retort for precooking under steam pressure.

As described in the foregoing paragraph, this vacuum-rotary dehydrator represented a type rather well known in industry. However, in consequence of a suggestion by one of the authors (William E. Gray) a means was added of rapidly removing moisture vapor during the early stages of the processing. It consisted of a small, finned heater, supplied with steam up to 20 pounds' pressure per square inch, and a small "squirrel cage" fan of conoidal type driven by a one-half-horse power, direct-connected motor. This unit was so arranged that it forced 750 cubic feet of heated air per minute through one-half of the open port on the top of the machine while the meat was being precooked. This resulted in forcing an equal volume of moisture-laden air per minute out through the other half of the open port. After cooking was completed the heater-fan unit was disconnected and the cover plate put over the opening. The vacuum pump was then started and the meat dehydrated under 29 inches of vacuum.

Vacuum-rotary Dehydrator B (Fig. 4) was made of steel and was steam jacketed on the bottom. The machine was of strictly laboratory size, with a capacity of 40 pounds of raw meat. Steam pressure in the jacket could be varied up to 20 pounds. The top of the machine could be left off or replaced after filling and the meat precooked at atmospheric pressure or at higher or reduced pressures, as desired. In any case the dehydration proper was carried out under vacuum, for which 29 inches could be furnished through a two-inch pipe connected with the pump.

The main difference between this machine and the vacuum-rotary Dehydrator A was in the agitator. In the case of Machine B the stirring device was of curved "sigma" shape and, moreover, was internally heated by steam. The clearance between the agitator as it revolved and the internal surface of the drying chamber was very close, not more than 0.01 inch. Means for determining the temperature of the meat during the processing was provided by a dial thermometer that was slipped through the journal and revolved with the "sigma" agitator. The entire dehydration chamber could be tilted forward for ease in removing the finished product.

Still another machine of this type was used in the investigations. It was identified as vacuum-rotary Dehydrator C (Fig. 5). The shape of the drying chamber was hexagonal and five of the six sides were steam jacketed, the cover accounting for the other side. The jacket could withstand as much as 30 pounds of steam pressure. The entire chamber revolved around its axis, the result being that the product was tumbled over and over or cascaded from one flat surface to another. Through use of the same vacuum equipment as employed with Machines A and B, a 29-inch vacuum could be created and maintained in Machine C.

The capacity of vacuum-rotary Dehydrator C was about 20 pounds of raw, ground meat. After precooking, the actual drying was done as the second phase of the process without transferring the product to another machine.

Still another vacuum-drying machine was included in the investigations. It was a vacuum-plate drier (Fig. 6) equipped so that brine at a temperature of approximately —15°C.(5°F.) or water or steam at higher

FIG. 3. Vacuum Dehydrator A being filled with ground, raw meat to be cooked and dried. The auxiliary attachment at the top center is a combination heater and blower for forcing heated air into the drier during the first or precooking stage of the operation.

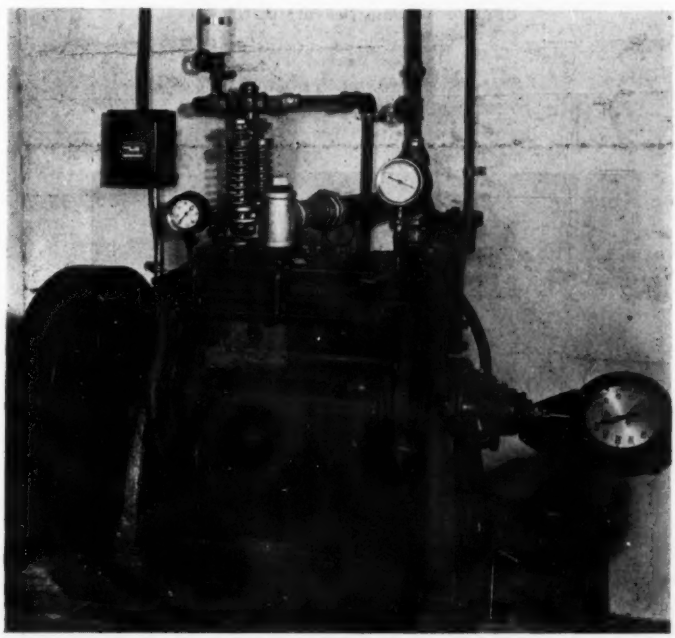

FIG. 4. Vacuum Dehydrator B.

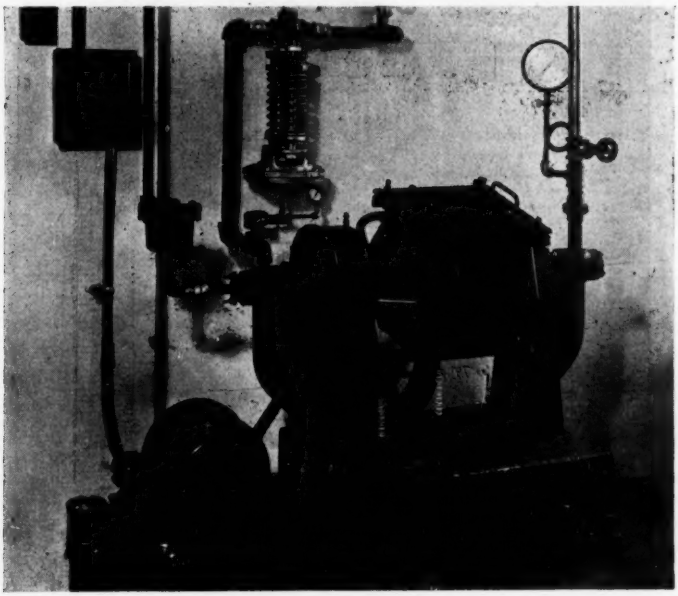

FIG. 5. Vacuum Dehydrator C.

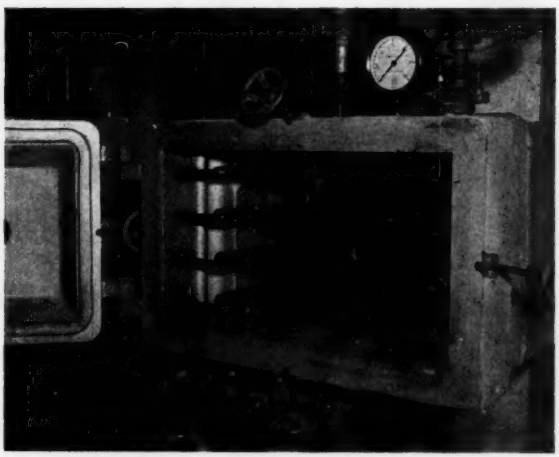

FIG. 6. Vacuum-plate dehydrator, showing interior. Note the four hollow plates or shelves.

temperatures could be introduced into the hollow plates or shelves. The machine had a total of four plates, each two feet square. In operation the raw or precooked meat was spread on shallow pans which were in turn placed on these hollow plates. A wide range of temperature and a vacuum as high as 29 inches could be obtained in the drying chamber. Moreover, the temperature could be changed as desired during the drying operation. By alternating 5°F. brine and steam periodically in the plates dehydration was even carried on by sublimation and evaporation succeeding each other.

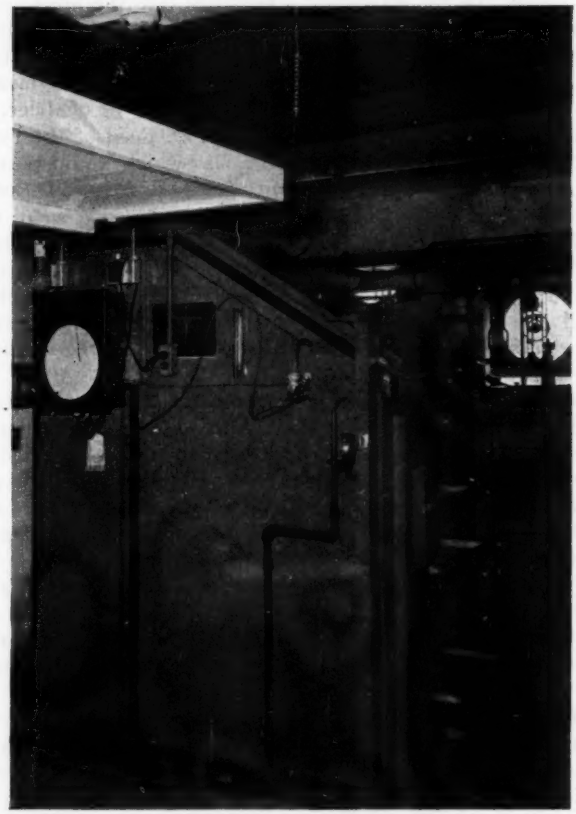

FIG. 7. Cabinet Dehydrator A.

AIR DEHYDRATORS

Cabinet Driers: Cabinet driers have a drying chamber holding one or more stacks of trays supported on angle-iron runners or a truck. Three machines of this type, differing in design and size, were used in the investigations. They were identified as cabinet Dehydrators A, B, and C; Machines A and B are illustrated (Figs. 7 and 8), respectively.

The trays were loaded with approximately 1.5 pounds of pretreated meat per square foot of surface and placed in the drier. Air was blown by a fan through the heaters across the trays and recirculated. Means for exhausting enough of the moist air through a damper and drawing in fresh air through another damper to maintain the desired relative humidity were provided. Automatic temperature controls were used to regulate the temperature. The relative humidity was maintained by the wet bulb actuating a compressed-air diaphragm motor, which alternately opened and closed the fresh-air and exhaust-air dampers, as well as the valve on a steam line for introducing moisture into the air when desired.

Cabinet Dehydrator B was provided with vanes and ducts for flowing the air across, up through, or down through the trays of meat. Thus it was possible to simulate the action and effect of drying by means of the endless belt, continuous conveyor, or moving-trays type of drier.

Cabinet Dehydrator C was designed by J. O. Reed of the Department staff. Features of this machine included a size which would permit operation on a semicommercial or pilot-plant scale. Moreover, because of the design, it was possible also to conduct experiments with small quantities of material. This flexibility of operation was in part obtained by providing means for varying the speed of the fans, and thereby the amount of air used, and by placing a curtain on the side of the drying chamber to control delivery of the air to the drying trays. This curtain made it possible to confine the air flow to any area occupied by the trays by blocking off the air inlet above the top tray.

Additional features were the positive control of the humidity of the air by recirculation and by admission of live steam. The discharge and intake of the air were controlled positively by motor-operated dampers automatically controlled by a temperature regulator. The exhaust of the moist air was continuous but the amount of air exhausted or introduced was only that required to maintain a uniform, predetermined temperature.

It should be possible to join a group of these units together to form a tunnel dehydrator which would give control of air conditions in all sections of the tunnel.

In the laboratory at Beltsville only a few runs, somewhat preliminary in nature, were made with this machine because of time limitations in the emergency meat-dehydration project.

The velocities of air flowing over the trays in cabinet Dehydrator A, when it was operated empty with the fan blowing the air first through the heater, then over the trays, and recirculating up through the return duct, were as shown (Table 1). Operating with a baffle covering the top four trays and the bottom four trays the velocities were as shown (Table 2).

Air-Rotary Dehydrators: Two types of air-rotary driers were subjected to some study in the investigations. However, one of them, identified as air-rotary Dehydrator A (Fig. 9), made much less use of air as the drying medium than the other. In the former instance heat was furnished by means of steam tubes running longitudinally through the cylinder and attached to the inner circumference by spacer rings. The meat came in direct contact with the hot tubes or pipes and tumbled from one to another as the drier revolved. The meat to be dried was fed

FIG. 8. Cabinet Dehydrator B.

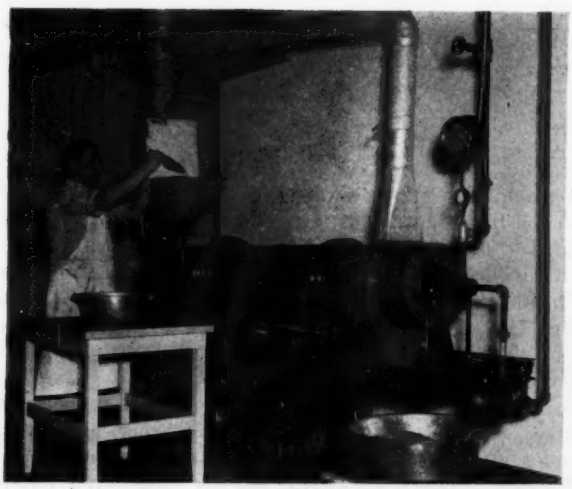

FIG. 9. Air-rotary Dehydrator A.

into one end of the cylinder and gradually worked its way to the other, where it was discharged. The time required for the meat to go through the drier was determined by the pitch or angle of declination on which the cylinder was set, as well as the speed of rotation and rate of feeding. A slight movement of air through the drier was created by a small exhaust fan. The cylinder was six feet long and 12 inches in diameter.

TABLE 1

Velocities of Air Over Different Trays in Cabinet Dehydrator A

Tray No.	Velocity of air
	ft./min.
1	Not sufficient room for anemometer
2	292
3	216
4	211
5	186
6	189
7	233
8	230
9	246
10	257
11	313
12	300
Below No. 12	33
Mean for 11 trays	243

TABLE 2

Velocities of Air Over Trays 5, 6, 7, and 8, When All Others Were Covered by Baffles

Tray No.	Velocity of air
	ft./min.
5	385
6	479
7	420
8	455
Mean for 4 trays	435

Another machine of this general type, well known in the industry and identified in the investigations as air-rotary Dehydrator B (Fig. 10), was heated by a blast of hot air being blown into the cylinder. The cylinder, which was 18 inches in diameter and 10 feet long, was made up of two shells, one inside the other. The inner shell was composed of a series of louvers (Fig. 11), and the air, heated to 148.9°C.(300°F.), was blown at the rate of 400 cubic feet per minute into the lower sealed-off segment at the end of the cylinder between the two shells so that it passed up through the narrow openings toward the center of the cylinder. In so doing it came in contact with the meat. The moist air was drawn out of the cylinder at the discharge end of the machine and recirculated to the desired extent. There were dampers for exhausting humid air and taking in fresh air to maintain a balanced proportion of fresh to wet air.

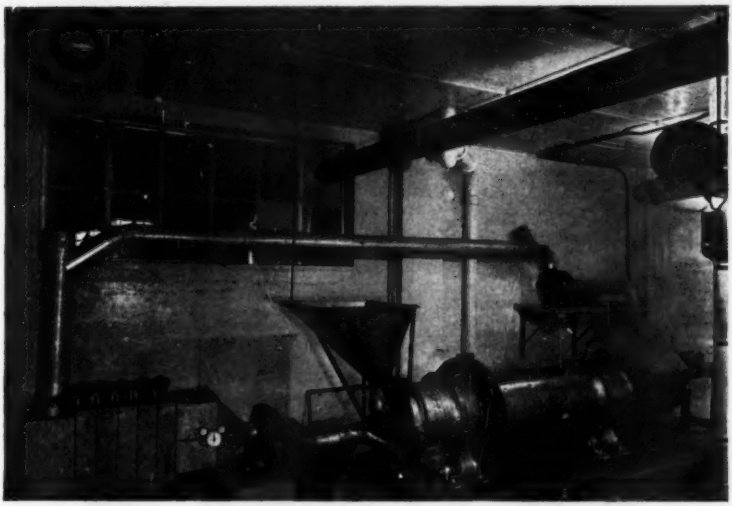

FIG. 10. Air-rotary Dehydrator B, showing heaters on left, feeding hopper in center, rotating cylinder on right center, collecting apparatus for dehydrated meat on right, and air discharge and recirculation ducts above.

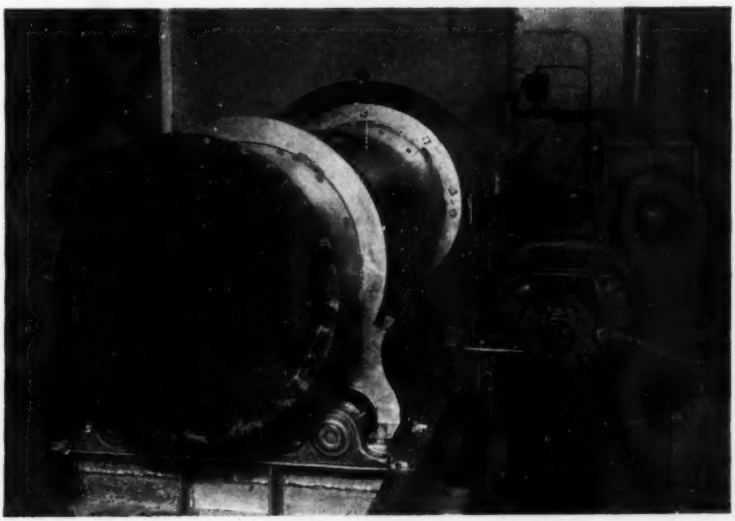

FIG. 11. Discharge end of air-rotary Dehydrator B with rings removed, showing louvers.

The meat was fed into the "wet end" of the cylinder and tumbled about while it worked its way toward the "dry end" where it was discharged. Temperatures of the meat throughout the length of the drier were read by use of thermocouples connected to a potentiometer.

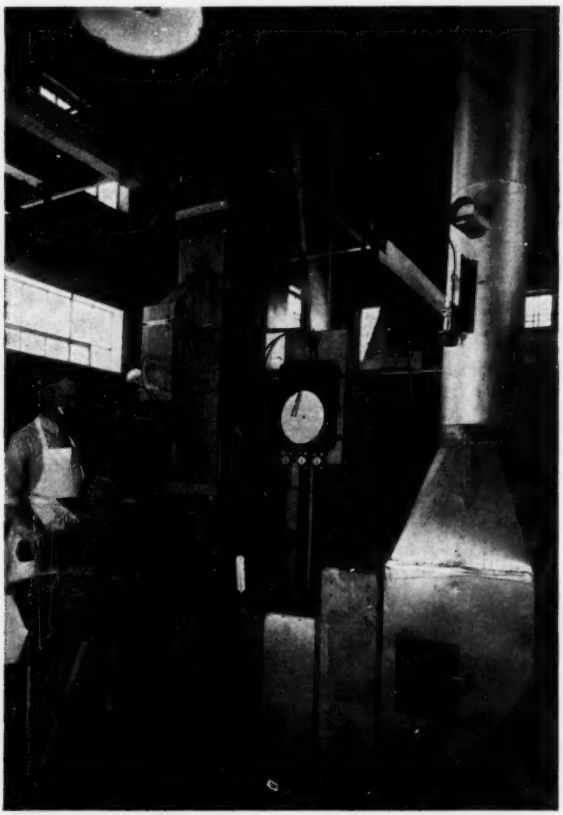

FIG. 12. Feeding precooked meat into the air-flotation dehydrator. The heater and fan are in the right foreground and the flotation chamber or stack is shown in the left center.

Provision was made for effective cleaning by attaching a cover plate at each end of the cylinder. With these plates in place the machine would retain hot water and washing powder while being rotated.

Air-Flotation Dehydrator: Embodying a principle used in certain other fields of drying, a dehydrator was designed by W. A. Noel of the Department staff in 1942 for use in the meat investigations. It made use of the lifting power of a vertical current of air to float meat in a chamber until the water was evaporated and was termed the "air-flotation" dehydrator.

The apparatus, largely shown (Fig. 12), consisted of a steam radiator for heating the air, a direct-connected, standard exhaust fan, the flotation chamber, a cyclone separator, connecting-duct work, and steam and electric supply lines.

Precooked meat was fed into the chamber at the top of the fan outlet, where it was caught by the air and carried upward. The theory was that this movement would continue until, owing to diverging side walls of the chamber, the lifting properties of the air would be so reduced that the meat would drop back a short distance and then be caught in another current and blown upward again. It was believed this alternating movement should continue until the moisture had largely disappeared, and the meat had become so light that it would float over into the collector.

The separator, circular in design, tapered to a small outlet at the bottom. Another opening, on the side near the top, received the air carrying the dried meat. On entering the separator, the meat-laden air was given a spinning motion, and centrifugal force threw the meat onto the side walls. As it lost velocity the meat moved down and out of the collector.

Through an opening at the top of the collector the air passed into a duct which returned a portion of it to the heater and fan. Just before the recirculated air entered the fan, fresh air was introduced through an automatically regulated damper and mixed with it. A hygrostat in the outlet air duct controlled the operation of the damper.

As described above, the dehydrator was operated on the batch basis, with a capacity of 50 pounds of fresh meat. It was recognized that to have the method entirely practical, however, it was probably necessary to have the machine operate as a continuous drier; therefore it was redesigned. Provision was made for three flotation stacks with their correlated separators which discharged their products into the succeeding flotation stacks. There was to be a constant continuous feed of wet material and discharge of dried product. However, at the time the emergency project was terminated this drier was not completed and evaluated.

PACKAGING ROOM AND EQUIPMENT

Recognition was given to the possible absorption of moisture by the dehydrated meat while it was being packaged. To obviate this possibility a room approximately 13 feet square and eight feet high was provided, with moderately good insulation including double windows. The room was placed under temperature control by the use of a pneumatic thermostat to hold the temperature at 22.2° C. (72° F.) ± 1° F. Humidity was controlled by the use of a fan-operated desiccating unit handling 1,000 cubic feet of air per minute. Commercial calcium chloride in two-inch blocks was the desiccating agent. Relative humidity was set for 30 per cent and a hygrostat on the unit started and stopped the fan. Located in the room were a vacuum can-sealing machine equipped for gas packing and facilities for packaging in other types of containers.

VITAMIN C AND CAROTENE IN ROSE HIPS AND PRODUCTS[1]

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The small fruits or berries formed after the flowering of roses are generally referred to as rose hips. The hips from wild roses have long been used for food and medicinal purposes in European countries, but only recently and with the advance of knowledge of vitamins has it been known that wild rose hips are an excellent source of vitamin C and also high in carotene or provitamin A. Wild roses grow in abundance in many places in this country and their value in foods has only recently received much attention.

Garden varieties of rose hips are low in vitamin C as shown by Gronau (1941) and Tuba, Hunter, Hutchinson, and Kennedy (1943). Wild rose hips are generally high in vitamin C but differ according to variety, location of growth, and maturity. Tuba *et al.* (1943) in Canada found hips of *Rosa laxa* to have 3,000 to 4,000 mg. per cent and *Rosa acicularis* 1,800 to 3,500 mg. per cent of vitamin C on a dry basis. Vadova, Beider, and Yanishevskaya (1941) and Vadova, Menshikova, and Yanishevskaya (1941) in Russia found that *Rosa cinnamomea* and *Rosa rugosa* ripen early and rank high in vitamin C potency of from 2,275 to 6,977 mg. per cent, while *Rosa canina* ripens late and is lower in vitamin C of from 711 to 1,338 mg. per cent. Wokes, Johnson, Organ, and Jacoby (1943) and Wokes, Johnson, and Duncan (1943) prepared dried extracts of hips from *Rosa canina* and *Rosa dumetorum* which contained 1,300 to 1,500 mg. per cent of vitamin C, 6,000 I. U. of provitamin A (carotene) per 100 grams, and 520 S. L. units of vitamin P per gram, but they found no significant amount of vitamin B_1. Randoin and Gallot (1940) found that the pulp of fresh *Rosa canina*, apparently grown in France, contained 743 to 1,340 mg. per cent of vitamin C. Schroderheim (1941), writing in a Swedish publication, found that the vitamin C content over a three-year period averaged 250 mg. per cent in *Rosa multiflora* and 1,180 mg. per cent in *Rosa nipponensis* of the dried pulp. Bailey (1941) reported in a Connecticut Agricultural Experiment Station bulletin that *Rosa rugosa* hips contained 22 mg. per cent and *Rosa multiflora japonica* only four mg. per cent of vitamin C.

Pyke and Melville (1942) found that rose hips in Scotland and northern England were higher in vitamin C than those found in southern England. Sabalitschka and Michels (1942) observed that the vitamin C content of rose hips increased as they ripened and then decreased upon overripening. The decrease upon storage was greater, the riper the hips at the time of harvest.

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Wokes, Johnson, Duncan, Organ, and Jacoby (1942) and Wokes, Johnson, Organ, and Jacoby (1943) found that a rose-hip sirup loses about 50 per cent of its vitamin C content from fall to spring, but this loss can be minimized by storing in nitrogen-filled containers at low temperatures and by concentration of the sirup. Charley and Pollard (1943) prepared a spray-dried powder from rose hips which contained 979 mg. per cent of vitamin C. The powder was gas-packed in cans and after three months' storage at room temperature showed a loss of about three per cent of the vitamin. Mikhov (1941) studied the possibilities of making rose-hip flour as a source for vitamin C.

Wild roses grow in abundance in many parts of the United States and the hips have not been utilized for food very extensively in this country. In view of the high vitamin C and provitamin A or carotene content found in wild roses grown in other countries it was of interest to make a study of rose hips grown in northern Idaho as well as juice and jam prepared from them.

EXPERIMENTAL PROCEDURE

Vitamin C determinations were made according to the colorimetric method, using 2,6-dichlorophenolindophenol described by Loeffler and Ponting (1942). Carotene determinations were carried out by the method described by Bolin and Khalapur (1938). Average hip weight and percentages of meat and seed were determined by using 50 rose hips. The moisture content was determined by drying them to constant weight in an air oven at 105°C.(221°F.).

The hips were collected from two different rose plots in this locality, one of which had small hips and the other much larger hips as representing typical growing species. A botanist identified the small hips as belonging to *Rosa spaldingii* and the large to *Rosa megalantha*. The hips were collected and analyzed the same day.

Analyses of Hips: The vitamin C and carotene content of the two species of rose hips as collected at different stages of maturity are shown (Table 1); data on average hip weight, moisture content, and average percentages of meat and seed are also included.

Effect of Frozen Storage: A large quantity of hips were collected and within a few hours were packed in sealed quart Mason jars and placed in a cold-storage locker maintained at —20.6°C.(—5°F.). Samples were analyzed at various intervals up to six months of storage. The results (Table 2) indicate little or no loss of vitamin C during this period.

Rose-Hip Jam: One pound of rose hips were added to three cups of boiling water and allowed to simmer for 30 minutes. The seeds were removed by using a strainer and the resulting purée was made up to four cups. This pulp was used for jam by following an ordinary recipe for raspberry jam as supplied by various pectin manufacturers. With use of some commercial pectin brands this jam will set at once while with others several weeks are required. The jam was stored in small glasses under wax cover in an ordinary fruit cellar and it was used for analyses at intervals (Table 2). The original vitamin C content was very high and the loss after six months was very small.

The jam had a rust-red color and the flavor was pleasant and fruity and characteristically its own, but many persons thought it resembled raspberry flavor.

TABLE 1

Data on Rose Hips Picked From Two Plots on Different Dates

Factors	Unripe	*Rosa spaldingii*		*Rosa megalantha*	
		Ripe		Ripe	
Date collected	Aug. 2	Aug. 26	Oct. 12	Aug. 26	Oct. 12
Color	Green	Orange-red	Red	Orange-red	Red
Average hip weight (gm.)	0.61	0.96	0.71	2.30	1.50
Moisture (pct.)	64.7	64.7	56.9	67.8	59.4
Meat (pct.)	67.8	62.8	65.5	75.0	69.8
Seed (pct.)	32.2	37.2	34.5	25.0	30.2
Vitamin C (mg./100 gm.)					
Whole hip	539	694	901	1124	783
Meat	796	1105	1375	1498	1122
Carotene (mg./100 gm.)					
Whole hip	11.1	19.0	25.2	28.2	23.4
Meat	16.4	30.3	38.3	37.6	33.5

Dehydration of Rose Hips: Some rose hips were dehydrated at 65.6°C. (150°F.) in an ordinary home dehydration unit with and without blanching, but 80 per cent or more of the vitamin C was lost by such treatments.

Vitamin C of Juice and Purée: A juice was made by taking one pound of hips to make four cups of juice as previously described. This juice was diluted with an equal volume of water and stored in a refrigerator at 5°C. (41°F.) in a covered milk bottle. The original vitamin C content of this juice was 194 mg. per 100 ml. and after eight days in the ice box it contained 161 mg. per 100 ml. This juice can be used as a drink either sweetened, with salt, or flavored with other fruit juices. It looks very much like tomato juice.

TABLE 2

Effect of Storage of Frozen Rose Hips and Jam Made From Them on Vitamin C Content

Storage time	Vitamin C	
	Frozen hips	Jam
mo.	*mg./100 gm.*	*mg./100 gm.*
Fresh	923	120.1
1	962	110.7
2	934	119.6
4	952	125.5
6	915	106.3

Purée obtained before water was added contained from 400 to 450 mg. of vitamin C per 100 ml. depending upon the hips used. If stored in the cold there is very little loss in vitamin C for several days.

DISCUSSION AND SUMMARY

The results shown (Table 1) indicate that as the rose hips ripen there is a decrease in moisture content and concomitant decrease in hip weight.

The seeds represented about one-fourth to one-third of the weight of the fresh hips. The green hips of *Rosa spaldingii* were somewhat lower in vitamin C and considerably lower in carotene than the ripe hips. The results of both vitamin C and carotene indicate that hips from *Rosa megalantha* ripen at an earlier date than those from *Rosa spaldingii*. The hips analyzed varied from 694 to 1,124 mg. per cent of vitamin C and 19 to 28 mg. per cent of carotene. On the basis of a daily requirement of 75 mg. per day of vitamin C for adults this amount would be supplied by eight to 12 rose hips of the type analyzed.

The jam was unusually high in vitamin C and stored satisfactorily for six months; the flavor was very well accepted by everyone who tasted it. A rose-hip purée could well be used to supplement the vitamin C content of other fruit juices in making jam and would perhaps produce pleasing flavor effects.

The juice as prepared was higher in vitamin C than tomato juice or orange juice and could well be employed as a source of vitamin C by using it sweetened or otherwise flavored to suit. Frozen hips could furnish such juice throughout the year.

Most of the vitamin C was lost by dehydration of rose hips in a home dehydration unit.

The use of rose hips in various foods, such as fruit soups, sherbets, juices, jams, and jellies, should be valuable owing to their high vitamin C and carotene content. Investigations into the culinary aspects of preparing rose hips should lead to many new and interesting ways of utilizing them.

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STABILITY OF ADDED VITAMINS IN BEER

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The stability of vitamins in solution depends upon several factors, such as temperature, pH, oxidation, and exposure to light. Appreciable quantities of several B-complex vitamins, with the exception of thiamin, have been found in fermented malt beverages by Laufer, Schwarz, and Laufer (1942). The possibility of increasing the vitamin content by improved malting and brewing methods, makes it desirable to know the effect of varying conditions of storage on the stability of water-soluble vitamins in beer.

Novak and Adams (1943) added definite amounts of thiamin, riboflavin, and niacin to separate portions of 86-proof whiskey in both clear and amber bottles and exposed them to daylight. They found no loss of thiamin or niacin in either bottle, but 50 per cent of the riboflavin was destroyed in the clear-glass bottle at the end of the first week, and within two months the amber bottle and control bottle, kept in the dark, had both lost 50 per cent of their riboflavin.

Evidently the action of light was primarily responsible for the rapid loss in the clear bottle, while less obvious factors must be taken into account to explain the change in the other bottles. In either case, the destruction of riboflavin was probably not accelerated by any chemical change in the whiskey itself. The high alcoholic content and minute amount of residual matter account for its stability.

Beer, a beverage of low alcoholic content, contains complex extractives including carbohydrates and protein derivatives. These are subject to chemical change if the bottled beer is stored under unfavorable conditions such as were chosen for part of this work. This was done to gain a comparison of the stability of certain vitamins in solution under normal and abnormal storage conditions.

EXPERIMENTAL PROCEDURE

The natural vitamin content of beer was supplemented with thiamin, riboflavin, and niacin. These vitamins are important in human nutrition and are quite stable in acid solution when subjected to a pasteurizing temperature of 60°C.(140°F.).

Measured amounts of beer of approximately four per cent alcoholic content by weight were drawn into three stainless-steel quarter barrels from a large storage tank. The first barrel was fortified with thiamin on the basis of 0.425 mg. per 12 fluid ounces (355 ml.), this being the liquid content of a standard beer bottle. To the second barrel riboflavin was added in the amount of 0.915 mg. per 12 fl. oz., and the third barrel received niacin on the basis of 19.5 mg. per 12 fl. oz. Each vitamin had

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previously been dissolved in a small amount of beer. The barrels were filled with carbon dioxide before receiving the beer in order to maintain the same carbon dioxide content in the experimental bottles as that found in those from the regular bottling line. They were thoroughly shaken three times at 30-minute intervals and left to stand several hours for the foam to subside. The beer was drawn into both clear and amber bottles that were capped and pasteurized in the usual manner.

All the clear bottles and a series of amber bottles from each barrel were placed on a window ledge facing north. Another group of amber bottles was stored in the dark, also at room temperature, while the fourth group was placed in a 4.4°C.(40°F.) refrigerator. Riboflavin assays were made on separate bottles at weekly intervals for 12 weeks. Thiamin and niacin assays were run each month for six months.

ASSAY METHODS

Thiamin was determined by a modification of the original Jansen (1936) thiochrome procedure. The fluorescence was measured in a Coleman Model 12 Electronic Photofluorometer.

A modification of Ferrebee's (1940) method was used for riboflavin analysis. The fluorescence produced after treatment with potassium permanganate and hydrogen peroxide was also read in the photofluorometer.

Niacin values were determined by the microbiological method of Snell and Wright (1941).

RESULTS

The natural thiamin content of the beer was found to be 0.023 microgram per ml. or 0.008 mg. per 12 fl. oz. bottle. This amount plus the 0.425 mg. added, made a total of 0.433 mg. thiamin actually contained in 12 fl. oz. of beer.

TABLE 1

Thiamin Content During Six Months' Storage

Storage time	Clear bottle on ledge	Amber bottle on ledge	Amber bottle in dark	Amber bottle in refrigerator
mo.	*mg.*	*mg.*	*mg.*	*mg.*
Amount at start	.433	.433	.433	.433
1	.40	.42	.43	.41
2	.38	.44	.42	.42
3	.37	.40	.41	.44
4	.35	.43	.43	.42
5	.33	.42	.45	.45
6	.31	.42	.44	.43

The table shows that a gradual loss of thiamin occurred in the clear bottles exposed to daylight until, at the end of six months, the loss had amounted to 29 per cent of the original content. There was no loss of thiamin in the other three storage groups.

The beer contained 0.115 mg. of riboflavin per 12 fl. oz. before fortification, making a total of 1.03 mg. per 12 fl. oz. bottle after fortification.

Fifty per cent of the riboflavin in the clear bottles was destroyed the first week. A more gradual decline then took place until at the end of 12

TABLE 2

Riboflavin Content During 12 Weeks' Storage

Storage time	Clear bottle on ledge	Amber bottle on ledge	Amber bottle in dark	Amber bottle in refrigerator
wk.	*mg.*	*mg.*	*mg.*	*mg.*
Amount at start	1.03	1.03	1.03	1.03
1	0.53	1.01	1.02	1.01
2	0.49	0.96	0.98	1.01
3	0.45	0.89	0.96	0.99
4	0.38	0.86	0.93	0.97
5	0.30	0.82	0.92	0.95
6	0.25	0.79	0.90	0.92
7	0.23	0.78	0.89	0.91
8	0.19	0.76	0.88	0.89
9	0.18	0.74	0.84	0.88
10	0.16	0.71	0.82	0.85
11	0.15	0.64	0.80	0.84
12	0.15	0.62	0.79	0.80

weeks, 85 per cent had been lost. The other three groups show less rapid destruction. The amber bottles on the shelf lost 40 per cent of their riboflavin, while those stored in the dark and in the refrigerator lost at very nearly the same rate, namely 23 per cent of their original content. Storage in the dark at room temperature was just as effective as a 40°F. refrigerator for riboflavin conservation.

The natural niacin content of the beer was 1.81 mg. per 12 fl. oz., making a total of 21.31 mg. per bottle after adding niacin.

TABLE 3

Niacin Content During Six Months' Storage

Storage time	Clear bottle on ledge	Amber bottle on ledge	Amber bottle in dark	Amber bottle in refrigerator
mo.	*mg.*	*mg.*	*mg.*	*mg.*
Amount at start	21.31	21.31	21.31	21.31
1	21.65	22.18	21.80	20.94
2	20.83	20.98	22.04	21.83
3	20.90	21.61	21.54	21.65
4	21.79	20.80	21.01	20.55
5	21.44	21.01	20.80	21.40
6	20.73	21.10	21.45	21.01

Niacin remained unchanged throughout the six months' test in all groups.

DISCUSSION

Soon after exposure, beer in the clear bottles on the ledge developed a characteristic "light-struck" flavor and odor. The amber bottles placed there gradually developed the same condition showing that amber glass only partially screens out the rays of light that accelerate this decomposition. Although these samples were not exposed to direct sunlight, they evidently underwent the same chemical changes as described by Gray,

Stone, and Rothchild (1941) in their work on the factors in beer responsible for the development of "light-struck" flavor. These authors state that sunlight causes complex sulphur compounds in beer to form traces of simpler sulphyhydryl bodies that are readily detected by their characteristic odor. The authors offer proof that these sulphur compounds tend to break down slowly even in the absence of light. However, air present in the bottle may oxidize the trace products formed before any odor becomes noticeable.

The samples of fortified beer kept in the dark and in the refrigerator were stored under conditions comparable to those that might be found during shipment and subsequent storage in commercial practice, except that the beer is usually consumed within a few weeks after being bottled.

The 29-per cent loss of thiamin in clear glass bottles stored on the ledge was probably due to the action of light on the vitamin itself. The beer in the amber bottles retained its original thiamin content.

As expected, riboflavin was rapidly destroyed in the clear bottles owing to its light sensitivity. It is somewhat unstable in solution even in the absence of light as indicated by the 23-per cent loss in both refrigerated and nonrefrigerated beer. Contrary to expectation, the difference in temperature had no effect on riboflavin conservation.

SUMMARY

Beer of four-per cent alcoholic content by weight was fortified with thiamin, riboflavin, and niacin. Clear and amber bottles containing each vitamin were placed on a window ledge and exposed to daylight. Another series of bottles was stored in the dark, also at room temperature, and a fourth group was kept in a 40°F. refrigerator.

At the end of six months, the clear glass bottles exposed to daylight had lost 29 per cent of their original thiamin content. There was no loss in the other groups.

Fifty per cent of the riboflavin in the clear bottles was destroyed the first week. This increased to 85 per cent within 12 weeks. During the same period, there was a 40-per cent reduction of riboflavin in the amber bottles on the window ledge and a 23-per cent loss in beer stored in the dark at room temperature and in a 40°F. refrigerator.

Niacin remained stable in all groups for six months.

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INFLUENCE OF SUPPLEMENTARY CALCIUM AND MAGNESIUM FERTILIZERS UPON NUTRITIVE VALUE OF KALE[1]

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It has been repeatedly observed that at times it is difficult, if not impossible, to produce experimental rickets in rats by feeding the Steenbock and Black (1925) rachitogenic ration. Since this ration contains 76 per cent yellow corn meal, Holmes and Tripp (1933) studied the influence of the composition of yellow corn on the effectiveness of a rachitogenic ration and found that the calcium-phosphorus ratio of samples of corn grown in the Midwest varied from 0.06 to 0.75 part of calcium to one part of phosphorus. In a review of papers published between 1844 and 1898 they found the calcium-phosphorus ratio varied from 0.017 to 0.230, and in papers published since 1909 the calcium-phosphorus ratio was found to vary from 0.04 to 0.45. These observations have raised questions concerning the effect of various factors upon the nutritive value of plants used for human food, and the present study was undertaken to accumulate data regarding the influence of supplementary calcium and magnesium fertilizers upon the dietary constituents supplied by kale.

For a long time kale *(Brassica oleracea)* has been used as a human food. It is classified as a green leafy vegetable and it is frequently considered with such plants as spinach, endive, lettuce, mustard greens, and turnip and beet tops. Since kale has relatively few insect enemies and can be grown on many types of soils, it is widely distributed throughout this country in home gardens and in commercial market gardens. Unfortunately its characteristic flavor is not relished by some people and thus it is not as extensively used as its food value justifies. However, if the high nutritive value of kale was more widely recognized by the average person, the amount consumed could be materially increased by using it in conjunction with lettuce, spinach, endive, Swiss chard, and other green leafy vegetables in mixed salads. Accordingly, in order to make a more complete evaluation of the nutritive value of kale possible assays have been made of kale which was grown with different amounts of calcium and magnesium.

EXPERIMENTAL PROCEDURE

Soil: The kale used in this study was grown on an alluvial soil reported to be a modified drift that accumulated from the waning ice sheet. This glacial outwash material, which is a feldspathic mica-schist, has been classified by Beaumont (1943a, b) as a Merrimac fine sandy loam that extends two feet or more below the ground surface. A more complete

[1]Contribution No. 545, Massachusetts Agricultural Experiment Station.

description of this soil has been reported by Holmes (1944) in a discussion of the riboflavin content of immature Massachusetts lettuce.

Fertilizer: The kale was produced upon four experimental plots of land that had been maintained under controlled fertilizer conditions for many years. For five years previous all four plots received a home-mixed 5-8-7 fertilizer at the rate of one ton per acre. The composition and constituents of this fertilizer have been reported in detail by Holmes and Crowley (1944). Since some of the constituents of this fertilizer were not available during the current season, a commercial 4-9-7 fertilizer was applied to all four plots at the rate of one ton per acre. For convenience the plots were designated A, B, C, and D. Plot A served as control. Plots B, C, and D received additional fertilizers as follows: Plot B received magnesium sulfate at the rate of 150 pounds per acre; Plot C, 150 pounds of magnesium sulfate and 1,000 pounds of limestone per acre; and Plot D, 1,000 pounds of limestone per acre but no magnesium sulfate.

Assay Procedures: The kale seed was sown directly on the experimental plots; 49, 51, 56, 59, and 63 days later typical well-developed leaves were picked from each plot at 8:00-8:30 A.M. when they were fresh and crisp. The leaves were washed to remove any adhering soil, freed of water, split to remove the midrib, and quickly cut into small pieces. Further disintegration was produced with the Waring blender using the appropriate liquid for the different assays. Since the kale was grown in close proximity to the laboratory, only about 20 minutes elapsed from the time the leaves were picked until the assays were commenced.

The assay methods of the Association of Official Agricultural Chemists (1940) were used to determine the water, calcium, iron, phosphorus, and magnesium content of the kale. The carotene content was determined by the chromatographic method reported by Wall and Kelley (1943).

DISCUSSION OF RESULTS

The detailed results of the assays of the 20 samples of kale are reported (Table 1); six determinations were made for each of the 20 samples and the volume of data obtained necessitates discussing them under separate headings.

WATER

The water content of the kale picked in the early morning ranged from a minimum of 80 per cent for the fifth sample from Plot B to 85 per cent for the first and third samples from Plot D. The average values were 83.2 for Plot A, 81.9 for Plot B, 81.8 for Plot C, and 83.9 per cent for Plot D. The water content of kale from Plots B and C, which received magnesium sulfate, was slightly lower than that from Plots A and D, which received no magnesium sulfate. The available data are altogether too meager, however, to permit any conclusion as to whether or not the ingestion of magnesium sulfate by plants causes removal of water from their tissues as occurs when magnesium sulfate is administered to animals.

These values are somewhat lower than 87.4 per cent, reported by Loughlin (1937), or 90.2, 92.7, 90.8, 91.2, and 90 per cent, reported by Davidson and LeClerc (1936), for five samples of kale from Virginia,

Maryland, and California. The water content of the kale assayed in this study is also less than 94.3, 95.1, 95.7, and 95.9 per cent found by Holmes and Crowley (1944) for Boston head lettuce grown on the same experimental plots at practically the same time.

TABLE 1

Influence of Supplementary Calcium and Magnesium Fertilizers Upon Composition of Freshly Picked Kale

Source	Age	Water	Calcium	Iron	Magnesium	Phosphorus	Carotene
	days	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Plot A	49	84.5	219.5	3.3	31.1	91.7	....
B	49	84.0	221.3	4.7	45.6	84.5	....
C	49	82.0	271.3	4.5	42.6	100.8	....
D	49	85.0	270.3	2.4	27.0	112.9	....
Plot A	51	82.0	217.7	3.4	32.5	87.9	6.1
B	51	82.0	205.4	2.3	45.6	93.2	6.1
C	51	81.0	318.2	1.8	38.5	119.6	5.9
D	51	83.0	357.6	2.3	25.3	110.2	7.0
Plot A	56	84.0	168.8	2.1	29.3	75.8	2.6
B	56	81.5	200.3	2.0	50.1	92.1	4.3
C	56	82.0	376.8	1.9	47.6	118.0	4.3
D	56	85.0	291.0	2.1	27.3	96.5	4.4
Plot A	59	83.0	205.5	2.6	33.2	81.2	6.2
B	59	82.0	220.1	3.7	50.2	79.2	5.9
C	59	83.0	293.3	1.9	37.1	102.5	5.3
D	59	84.0	297.7	1.9	24.1	98.1	5.6
Plot A	63	82.5	255.2	3.0	37.0	79.5	5.7
B	63	80.0	229.5	3.0	55.3	87.1	8.7
C	63	81.0	337.9	3.1	45.6	116.1	6.7
D	63	82.5	301.7	2.2	27.8	103.7	7.3
Averages							
Plot A	56	83.2	213.3	2.9	32.6	83.2	5.2
B	56	81.9	215.3	3.1	49.4	87.2	6.3
C	56	81.8	319.5	2.6	42.3	111.4	5.6
D	56	83.9	303.7	2.2	26.3	104.3	6.1

CALCIUM

The amount of calcium in the kale varied from 168.8 to 376.8 mg. per 100 gm. The average calcium content of kale was 213.3 mg. per 100 gm. for Plot A, 215.3 for Plot B, 319.5 for Plot C, and 303.7 for Plot D. The average values for calcium for Plots A and B, which received no supplementary calcium fertilizer, are only about two-thirds as large as the values obtained for Plots C and D, which received 1,000 pounds of limestone per acre. It appears from these data that the limestone caused a valuable increase in the calcium content of kale but the addition of magnesium sulfate alone did not increase the calcium content of kale significantly.

Loughlin (1937) found 0.291 per cent (291 mg. per 100 gm.) of calcium in fresh kale. Her assay is based upon the 55 per cent of the purchased material that remained after tough petioles and discolored or

bruised leaves had been discarded. Davidson and LeClerc (1936) obtained from 1.37 to 2.36 per cent of calcium from six samples of kale, dry basis (essentially 137 to 2.36 mg. per 100 gm. on a fresh basis), that represented different varieties, localities, soils, and fertilizer conditions. In a study of the availability of calcium from typical foods Fincke and Sherman (1935) concluded that the calcium of kale was nearly as well utilized as that of milk which is very frequently used as a standard in calcium utilization studies. Hence the application of limestone as a supplementary fertilizer enhanced the value of the kale as a source of readily assimilable dietary calcium.

IRON

The extreme values obtained for the iron in kale were 1.8 mg. for Plot C and 4.7 mg. per 100 gm. for Plot B. The averages computed for the four experimental plots were 2.9 mg. of iron per 100 gm. for Plot A, 3.1 for Plot B, 2.6 for Plot C, and 2.2 for Plot D. Since the amount of iron in the kale from Plots A and B was slightly larger than that from Plots C and D, one may conclude that the addition of limestone as a supplementary fertilizer tends to decrease the iron content of the kale; on the other hand, magnesium sulfate did not produce any significant effect.

In a study of the availability of iron in various foods Ascham, Speirs, and Maddox (1938) found 0.308 mg. of iron per gm. of kale (dry basis) and that it was 75 per cent ionizable. They also found that when anemic rats were fed enough kale to supply 0.3 mg. of iron per day the hemoglobin content of the blood increased 6.3 gm. per 100 c.c. Accordingly, if one eats generous amounts of kale at frequent intervals, it can contribute to the utilizable iron content of the human dietary.

MAGNESIUM

The minimum amount of magnesium found in the kale was 24.1 and the maximum 55.3 mg. per 100 gm. The average values obtained for the 20 magnesium assays were 32.6 mg. of magnesium per 100 gm. of kale for Plot A, 49.4 for Plot B, 42.3 for Plot C, and 26.3 for Plot D. The kale from Plots A and D, that received no magnesium sulfate, contained only about two-thirds as much magnesium as the kale from Plots B and C, which received magnesium sulfate as a supplementary fertilizer. Since the application of 150 pounds of magnesium sulfate per acre caused a 50-per cent increase in the magnesium content of the kale, it is evident that the magnesium sulfate fertilizer stimulated the kale to incorporate a larger amount of magnesium into its tissues. On the other hand, the application of 1,000 pounds of limestone per acre caused a 15-per cent decrease in the magnesium content of kale when applied either alone or in conjunction with the magnesium sulfate.

A review of the literature produced very little data concerning the magnesium content of kale. Davidson and LeClerc (1936) determined the magnesium content of six samples of kale (dry basis). Five samples were Dwarf Blue Curled Scotch kale obtained from various locations in Washington, D. C., Virginia, and Maryland. The kale contained 0.32, 0.29, 0.33, 0.26, and 0.24 per cent of magnesium. The sixth sample was Tall Scotch Curled kale grown on irrigated California land and contained 0.74 per

cent of magnesium. The larger amount of magnesium in this sample was probably due to the irrigation water containing 62 p.p.m. of magnesium.

PHOSPHORUS

The results of the phosphorus assays of the kale from the different experimental plots showed considerable variation in the amount of phosphorus in the kale. The smallest amount found was 75.8 mg. and the largest was 119.6 mg. of phosphorus per 100 gm. The average values for the four plots were 83.2 mg. of phosphorus per 100 gm. for Plot A, 87.2 for Plot B, 111.4 for Plot C, and 104.3 for Plot D.

The amount of phosphorus in the kale from Plots C and D is definitely larger than the amount found in kale from Plots A and B, and very strongly suggests that the limestone influenced this difference in phosphorus. Except for the first harvest period the values obtained for the phosphorus content of kale from Plot C were higher than corresponding values from Plot D. Thus it appears that the magnesium sulfate applied with limestone stimulated the production of kale with a higher phosphorus content than when only limestone was used.

Fresh kale, representing 55 per cent of the purchased material that remained after the tough petioles and discolored and bruised leaves had been discarded, was assayed by Loughlin (1937) who obtained 0.082 per cent of phosphorus. Davidson and LeClerc (1936) found 0.25, 0.56, 0.64, 0.78, 0.68, and 0.65 per cent of phosphorus in six samples of kale (dry basis). The water content of the first sample is not reported. The other five samples average 0.66 per cent of phosphorus and the fresh material contained an average of 91 per cent of water or the fresh kale contained 59.4 mg. of phosphorus per 100 gm., which is lower than the minimum value obtained in this study and definitely lower than the average value for the kale from any of the four experimental plots.

CAROTENE

The amount of carotene in the freshly picked kale varied from 2.6 to 8.7 mg. per 100 gm. The average amount of carotene in the kale from the different plots was 5.2 mg. per 100 gm. for Plot A, 6.3 for Plot B, 5.6 for Plot C, and 6.1 for Plot D.

The agreement between the average carotene contents of the fresh kale from the four experimental plots indicates that the magnesium sulfate and limestone used as supplementary fertilizers did not significantly affect the amount of carotene found in the kale.

It is interesting to compare these values with those reported by a number of investigators. Sherman and Todhunter (1934), in rat tests using carotene dissolved in peanut oil as a standard of reference, found kale contained 200 vitamin A units per gram and was one of the richest natural plant sources of vitamin A. Booher, Hewston, and Marsh (1941), in feeding experiments with rats, found the vitamin A potency of kale was 10,500 international units per 100 gm., which was similar to 10,700 international units that they obtained for carrots. In a later paper Booher, Hartzler, and Hewston (1942) included a European assay by the spectroscopic method that reported the vitamin A content of raw kale to be

9,870 international units per 100 gm. Adams and Smith (1944) cited the vitamin A content of Dwarf Curled kale grown in South Dakota as being 3,857 international units per 100 gm.

SUMMARY

In order to secure a more complete evaluation of the nutritive value of kale a study was conducted to accumulate data regarding the influence of calcium and magnesium upon the composition of kale. The kale was grown under known soil and fertilizer conditions with varying amounts of magnesium sulfate and limestone added as supplementary fertilizers.

The addition of 150 pounds of magnesium sulfate per acre definitely increased the magnesium content of the kale and may have slightly increased the calcium and phosphorus content. Limestone, applied at the rate of 1,000 pounds per acre, definitely increased the calcium and phosphorus content of the kale may have depressed its iron content. Neither magnesium sulfate nor limestone nor both produced any pronounced effect on the water or the carotene content of the kale. As compared with other leafy vegetables kale is rich in carotene, the precursor of vitamin A. Judged by the results of this study the use of magnesium sulfate and limestone as supplementary fertilizers enhances the food value of kale.

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RELATIONSHIPS OF CERTAIN CHEMICAL PROPERTIES OF SOILS NEAR MIDLAND, DOUGLAS COUNTY, KANSAS, TO YIELD OF OATS HARVESTED AT JOINTING STAGE[1]

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The increasing use of dehydrated, immature cereal grasses as a source of protein and vitamins for human food as well as for stock consumption makes the problem of the effects of soil on the growth of these species of especial significance. The vast literature concerning cultural conditions for cereals is largely based on studies of the factors which affect the yield and composition of the grain. Considerable attention also has been directed towards factors affecting the quality of cereals cut for hay, but since the extensive use of immature grasses as a source of protein and vitamins has been only recently developed, little is known, except by inference and by limited practical experience, concerning the effect of soil characteristics on their yields and food values when harvested at this early stage of growth. This is the first of a series of papers which will describe the effects of certain chemical characteristics of soils near Midland, Douglas County, Kansas, on the responses of cereal grasses. The present paper is concerned with the effect of these properties on the growth of oats as determined by the yield of dry matter when harvested at the jointing stage.

The growth of plants in the field occurs in an environment different from any possible artificially arranged conditions. However, if one attempts to compare the effects of different types of soil under field conditions, he is confronted with the choice between the comparison of a large number of soils which unavoidably involves significant variations in climate and other local conditions, or the limitation of the area to be studied in order to escape the complications involved in the comparison of data obtained from widely separated fields. It is obvious that the first choice is almost totally unsatisfactory since it involves a hopeless confusion of soil and climatic factors. The second choice is not wholly satisfactory, but at least, the smaller the area, the smaller will be the effects of climate and the more apparent will become the influence of the soil itself.

Further, it is essential in such a study as the one described in the present paper that the crops be planted and harvested at approximately the same time in order to lessen the effect of seasonal variation.

The difficulties described make it apparent that only a few types of soil can ever be included in a simultaneous comparison of the influence

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of their properties on plant growth under naturally occurring field conditions. However, the difficulties inherent in such a study should not discourage students from obtaining such data as are possible and attempting to identify the relationships of specific soil properties on plant response.

MATERIALS AND METHODS

The soils included in the present study may be separated into three major classes according to their fundamental properties described by Wynd and Romig (1943). The correct interpretation of the data presented in the figures depends on the recognition of these group differences. Fields 19 and 11E (Points 3 and 4) are clayey soils of very low base-exchange capacity, and consequently they contain small amounts of replaceable bases. Since they are calcareous, and have a high pH, they form a group terminal to the group represented by Fields 12, 14B, and 18 (Points 5, 6, and 7). These soils grade into each other, in so far as their properties pertain to the present study. On the other hand, Fields 2A and 2B (Points 1 and 2) are profoundly different from all the others because they contain unusually small amounts of replaceable bases for soils having such high base-exchange capacities. This results in a low degree of base saturation and a low pH. Since their base-exchange capacity depends largely on organic matter, which is itself nitrogenous, it follows that these soils are characterized by an unusually large nitrogen-replaceable base ratio. The importance of this condition for the growth of oats is discussed in a later section of the paper.

The crops were harvested just as jointing began, since at this stage of growth the tissue exhibits its maximum nutritive value on the percentage composition basis. The material was dehydrated in an Arnold hot-air dehydrator. Individual fields were 20 to 40 acres in extent and the entire yield was used in calculating the yield in pounds per acre. The yields of dry matter are presented (Table 1); the M.S.C. file numbers refer to the

TABLE 1

Yields of Oats, Cut at Jointing Stage, Grown on Various Soils in Vicinity of Midland, Kansas

M.S.C. file No.		Field No.	Point No.	Date harvested	Yield, per acre
Soil	Oats				
					lb.
267	315	2A	1	Oct. 8, 1940	221
268	317	2B	2	Oct. 11, 1940	304
269	319	19	3	Oct. 17, 1940	200
270	320	11E	4	Oct. 18, 1940	200
272	321	12	5	Oct. 21, 1940	389
274	318	14B	6	Oct. 23, 1940	253
280	325	18	7	Nov. 1, 1940	552

original samples preserved in the Laboratory of Plant Physiology at Michigan State College. Individual soils may be identified in the tables of the previous publication by comparing the file numbers of the soils.

The point numbers refer to data in the figures which enable the reader to identify the soil from which each individual yield was obtained.

EXPERIMENTAL RESULTS

Some of the effects of soil properties on the growth of oats are obvious from an examination of the figures, while others are not evident unless the figures are compared with each other. Figure 1 suggests that base-exchange capacity is very important in influencing yield. Points 1 and 2 are out of line, but this is explained by the data presented in Fig. 2. It is apparent from an examination of the latter that growth is remarkably dependent on the amount of total replaceable bases. In this figure, Points 1 and 2 fall into line which indicates that growth follows the amount of replaceable bases rather than the base-exchange capacity.

The degree of base saturation is shown (Fig. 3) to have no intrinsic importance, at least within the range of variation of the soils included in the present study. Low yields are associated with both high and low degrees of base saturation. The higher yields are obtained from soils highly saturated with bases, but this is because these soils contain more nutrients.

The pH, on the other hand, seems to be very slightly related to yield if the soils are separated into high and low pH groups; Fig. 4 shows that increasing pH is positively related to yield in soils of these groups. This is probably dependent on the increasing amount of total replaceable bases, especially calcium, which causes the higher pH. The pH itself probably is relatively unimportant.

The effects of the individual replaceable bases are clearly evident from Figs. 5, 7, and 9. The relationship of replaceable calcium is especially great. Figure 5 resembles Fig. 2 so closely that it is probable the effect of the total amount of bases is largely dependent on the calcium component. Magnesium is less definite in its effect on growth than is calcium. Figure 7 shows that Points 1 and 2 are out of line. Yields represented by Points 1 and 2 depended quantitatively on calcium but not on magnesium.

The eccentric locations of Points 1 and 2 (Fig. 7) resemble their locations in Fig. 1 for base-exchange capacity, Fig. 11 for loss on ignition, and Fig. 12 for nitrogen. These similarities show that the amounts of replaceable magnesium are very closely related to the complex of factors associated with the organic matter in these soils.

The relationship of replaceable potassium to the yields as presented (Fig. 9) is positive, even though the points representing individual yields are considerably scattered. Some of these irregularities are caused by inaccuracies in the determination of the very small amounts of potassium present. The positive trend of the relationship of replaceable potassium to growth, however, is evident.

The ratios of the individual bases to the total bases appear from Figs. 6, 8, and 10 to be inversely related to yield. If a ratio of nutrient ions to each other or to the total nutrients present be regarded as having an intrinsic importance, then it would not be possible for the greatest growth to result when *each* nutrient was present in smaller proportions to the

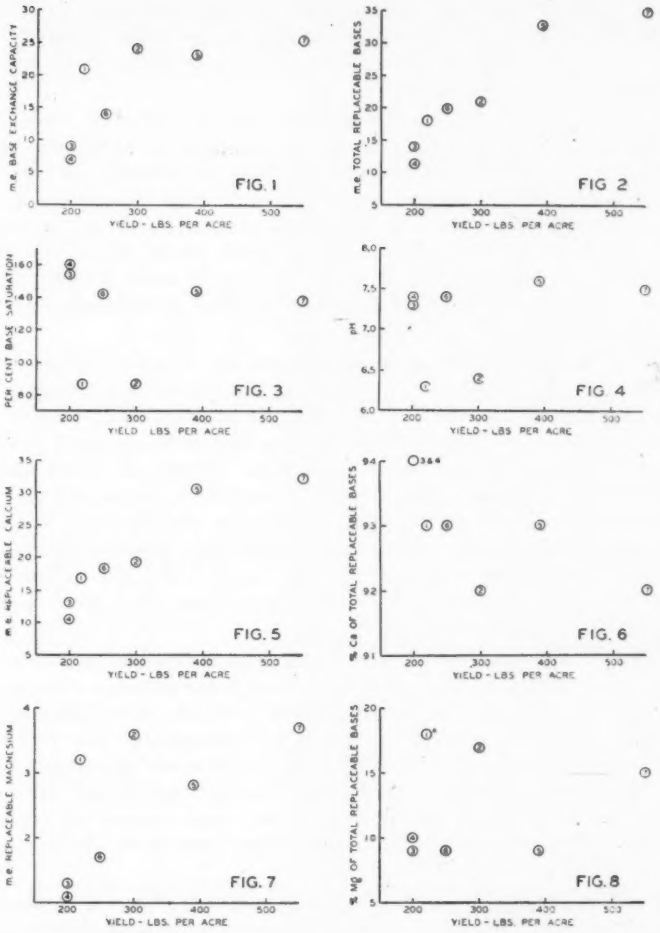

FIG. 1. Relationship between base-exchange capacity of soils and yield of dry matter of oats.

FIG. 2. Relationship between total replaceable bases and yield of dry matter of oats.

FIG. 3. Relationship between percentage of base saturation of soils and yield of dry matter of oats.

FIG. 4. Relationship between pH of soils and yield of dry matter of oats.

FIG. 5. Relationship between replaceable calcium in soils and yield of dry matter of oats.

FIG. 6. Relationship between ratio of replaceable calcium to total replaceable bases in soils and yield of dry matter of oats.

FIG. 7. Relationship between replaceable magnesium in soils and yield of dry matter of oats.

FIG. 8. Relationship between ratio of replaceable magnesium to total replaceable bases in soils and yield of dry matter of oats.

total bases. This would suggest that each ion was itself harmful, but that its toxic effect was overcome as the amounts of the other bases increased. But increasing amounts of each nutrient base are shown to be beneficial, even though in various degrees.

The effects of the different magnitudes of the ratios of each base to the total bases appear reasonable if we postulate a beneficial effect of each base. Then the yields would increase necessarily as the ratios diminished since the yield accompanying each ratio represents the yield increase due to a single base as compared with the sum of the increases due to all bases; for example, yields increase as the $Ca/Ca + Mg + K$ ratio lessens, even though Fig. 5 shows calcium to exert a very beneficial influence. This would be expected, since the effect of calcium alone is less than the total effects of all ions.

The amount of organic matter was shown by Wynd and Romig (1943) to govern the base-exchange capacity and the amount of nitrogen in the soils in the vicinity of Midland. Comparison of Figs. 1, 11, and 12 shows that the relationships of each of these three factors to the yield are almost identical since they are mutually dependent. Figure 12 is especially instructive since it shows that increasing growth accompanies increasing amounts of nitrogen in the soil only so long as the increases in nitrogen are accompanied by greater amounts of replaceable bases. The high nitrogen itself in fields represented by Points 1 and 2 did not induce greater growth. Figure 2 shows that the yields from these fields were dependent on the amount of replaceable bases.

The effect of weak-acid-soluble phosphorus is vaguely apparent. Figure 13 shows that the higher yields were from fields having a comparatively high amount of phosphorus, but since these fields also are characterized by many other favorable properties, the effect of phosphorus may be obscured because it is present in amounts which prevent it from becoming a limiting factor. For instance, Fig. 2 shows that yields from Fields 2A and 2B (Points 1 and 2) are closely dependent on their content of replaceable bases, and under these circumstances other nutritive factors are not limiting.

The effect of calcium carbonate is presented (Fig. 14); those soils which contain significant amounts of carbonate show a favorable response to increasing amounts. This effect, like that of pH, is dependent on the higher amount of replaceable bases which accompanies the carbonate content. It is important to point out that the carbonate values are expressed arbitrarily as the calcium salt although various amounts of other nutritive ions also are present as carbonates. Soils 2A and 2B (Points 1 and 2) do not contain significant amounts of carbonate.

A survey of all the data presented in the figures shows that the amount of replaceable bases is the most important factor in determining the yield of oats on the soils included in the present study. The replaceable calcium probably is the most important single component of the total replaceable bases. Potassium is of lesser importance than calcium. Magnesium appears to show a beneficial effect, although its influence is obscured by calcium and nitrogen which accompany it.

The chemical characteristics of soils in the vicinity of Midland that are most productive of dry matter of oats harvested at the jointing stage may be summarized as follows: base-exchange capacity more than 15 milliequivalents per 100 grams, total replaceable bases more than 20

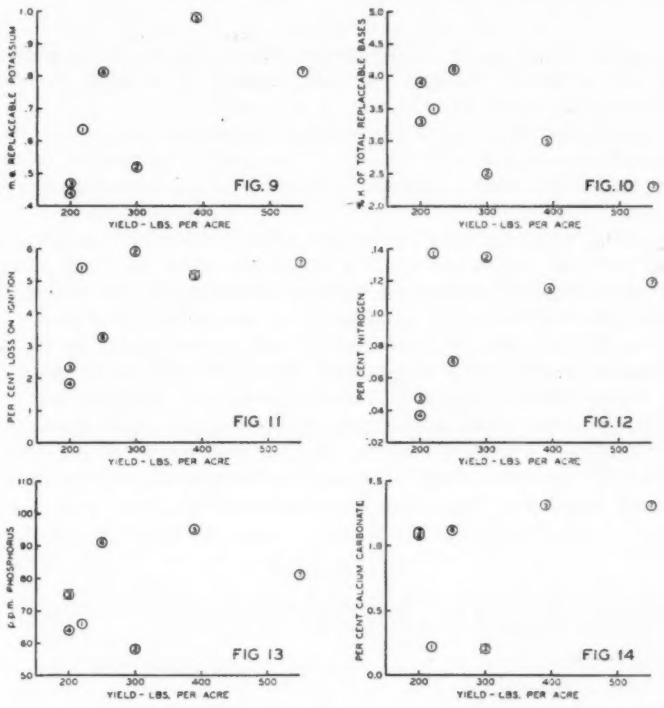

FIG. 9. Relationship between replaceable potassium in soils and yield of dry matter of oats.

FIG. 10. Relationship between ratio of replaceable potassium to total replaceable bases in soils and yield of dry matter of oats.

FIG. 11. Relationship between percentage loss on ignition of soils and yield of dry matter of oats.

FIG. 12. Relationship between nitrogen in soils and yield of dry matter of oats.

FIG. 13. Relationship between acid soluble phosphorus in soils and yield of dry matter of oats.

FIG. 14. Relationship between $CaCO_3$ in soils and yield of dry matter of oats.

milliequivalents, exchangeable calcium more than 18 milliequivalents, exchangeable magnesium more than 1.5 milliequivalents, replaceable potassium more than 0.5 milliequivalent, loss on ignition more than three per cent, and nitrogen more than 0.07 per cent. The pH, degree of base saturation, and carbonate content are unimportant within the limits of the values found in these soils. Soils containing less than 17 milliequivalents of total replaceable bases are unsatisfactory under the conditions described.

The limits of the chemical properties of productive and nonproductive soils are not permanent values. They depend on arbitrary standards of production, on the bacterial cycles of nutrient renewal in the soil, on the rate of weathering of the parent materials, and on the rate of loss by leaching and crop absorption.

SUMMARY

The yield of dry matter of immature oats grown on seven soils in the vicinity of Midland, Douglas County, Kansas, is related to chemical properties of the upper eight inches of the profile.

The total amount of replaceable bases is the most important chemical characteristic associated with variations in yield. The amount of calcium appears to be the most important single component of the replaceable bases.

Increasing values of base-exchange capacity, organic matter, carbonate, and pH also appear to exert a favorable effect on yield since they parallel the amount of replaceable bases under some, but not all, conditions.

Satisfactory soils for the production of immature cereal grass, in the vicinity of Midland, are characterized by soil properties having the following minimum values: total replaceable bases 20 milliequivalents per 100 grams, base-exchange capacity 15 milliequivalents, exchangeable calcium 18 milliequivalents, exchangeable magnesium 1.5 milliequivalents, replaceable potassium 0.5 milliequivalent, loss on ignition three per cent, and nitrogen 0.07 per cent. Soils containing less than 15 milliequivalents of replaceable bases per 100 grams are unsatisfactory.

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RELATIONSHIPS OF CERTAIN CHEMICAL PROPERTIES OF SOILS NEAR MIDLAND, DOUGLAS COUNTY, KANSAS, TO ACCUMULATION OF PROTEIN IN OAT LEAVES HARVESTED AT JOINTING STAGE[1]

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The chemical properties of a group of soils in the vicinity of Midland, Douglas County, Kansas, have been described by Wynd and Romig (1943), and the effects of certain of these properties on the growth of oats harvested at the jointing stage have been described by Wynd and Noggle (1945). The latter paper pointed out that the total amount of replaceable bases, as determined in the ammonium acetate leachate, was the most important chemical property of these soils in governing the growth of oats under the conditions studied. Variations in the amount of replaceable calcium were found to be the most closely associated with yield. So important were the replaceable bases that even the nitrogen content of the soil appeared to be secondary in its effect on yield.

The importance of immature cereal grasses as sources of protein and vitamins, for human consumption as well as for stock foods, depends on the high concentration of these substances in the leaves just before jointing begins. When cereals are grown for these purposes, the total yield per acre is secondary in importance to the composition of the tissue. Despite the extensive literature describing the culture of cereals, there is a surprisingly small amount of information available concerning the conditions which govern the accumulation of protein in the leaves at, or just before, the jointing stage. The present paper describes the relationships of the chemical properties of seven soils in the vicinity of Midland, Douglas County, Kansas, on the protein content of the leaves of oats.

MATERIALS AND METHODS

The determinations were made on samples taken from the same harvests which furnished the data for the study of the yield in dry matter previously reported by Wynd and Noggle (1945). These determinations therefore show the effects of the same soils that were included in the former study.

The total nitrogen was determined by the Kjeldahl method modified to include the nitrogen of nitrates as recommended by the Association of Official Agricultural Chemists (1935). The data (Table 1) are expressed in terms of percentages of the oven-dry tissue and as yields in pounds per acre. The M.S.C. file numbers refer to the original samples permanently on file in the Laboratory of Plant Physiology at Michigan State

[1] Expenses incurred by the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

College. The chemical data for each of the soils may be identified in the tables published by Wynd and Romig (1943) by referring to corresponding M.S.C. file numbers. The point numbers identify in the figures the soils from which each of the data was obtained.

The soils included in the present study fall into groups on the basis of their content of replaceable bases and degree of base saturation. Wynd and Noggle (1945) have emphasized the necessity of bearing in mind these major characteristics in interpreting the effects of specific chemical factors on the growth of oats. For the present discussion it is necessary to repeat only that Fields 11E, 12, 14B, 18, and 19 (Points 3 to 7, inclusive) are composed of soils which are calcareous, with a high pH, and a high degree of base saturation; and they vary from each other primarily in the amounts of replaceable bases which they contain. Fields 2A and 2B (Points 1 and 2) represent soils which have a moderately high content

TABLE 1

Protein Content of Oat Leaves, Harvested at Jointing Stage

M.S.C. file No.		Field No.	Point No.	Date	Dry matter, per acre	Total N	N yield, per acre	Crude protein (N x 6.25)	Protein yield, per acre
Soil	Oats								
					lb.	*pct.*	*lb.*	*pct.*	*lb.*
267	315	2A	1	Oct. 8, 1940	221	4.13	9.13	25.81	57.04
268	317	2B	2	Oct. 11, 1940	304	4.62	14.04	28.88	87.80
269	319	19	3	Oct. 17, 1940	200	3.01	6.02	18.80	37.60
270	320	11E	4	Oct. 18, 1940	200	3.52	7.04	22.00	44.0
272	321	12	5	Oct. 21, 1940	389	3.85	14.98	24.06	93.59
274	318	14B	6	Oct. 23, 1940	253	3.74	9.46	23.38	59.15
280	325	18	7	Nov. 1, 1940	552	4.47	24.67	27.94	154.23

of replaceable bases, but are not calcareous, and have a low pH and a low degree of base saturation. These soils have a comparatively higher nitrogen content and high base-exchange capacity owing to the presence of comparatively large amounts of organic matter. For further details the papers by Wynd and Romig (1943) and Wynd and Noggle (1945) should be consulted.

The replaceable bases were determined in the 1 N neutral ammonium acetate leachate. This extracting solution removes other bases from calcareous soils than those adsorbed on the colloids. This situation should be borne in mind in interpreting the data concerning the total replaceable bases, individual bases, and degree of base saturation.

EXPERIMENTAL RESULTS

Variations in the base-exchange capacity of the soils are shown (Fig. 1A) to accompany changes in the percentage of nitrogen in oat leaves. Comparison of Figs. 1A and 12A suggests that this effect is associated with the nitrogen present in the organic matter responsible for the major portion of the base-exchange capacity upon which depends, in many instances the amount of total replaceable bases. It is therefore difficult to interpret the cause of the effect of the base-exchange capacity. Since

the base-exchange capacity also is related positively to the yield of dry matter, the relationship between this factor and the yield of protein is especially obvious.

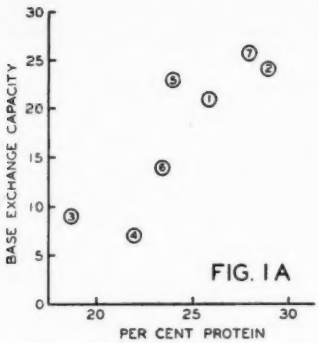
The amount of replaceable bases also appears to be related positively to the percentage of protein in the oat leaves. Points 1 and 2 (Fig. 2A) represent tissue containing a high percentage of protein even though the fields from which these samples came have only a moderate amount of replaceable bases. Comparison with Fig. 12A shows that these points fall well into line on the basis of their nitrogen content. It appears probable that much of the apparent effect of the total replaceable bases is due to the nitrogen content of the organic colloids which are responsible for the retention of these nutrients in the soil, since the effect of higher amounts of replaceable bases is positive only when the amount of nitrogen in the soil is also greater. The effect of the nitrogen becomes dominant over that of the bases when increases in the replaceable bases are not accompanied by higher amounts of nitrogen. It is important to note that this interpretation is based solely on a comparison of Figs. 1A and 2A with 12A. Since the amounts of total replaceable bases have a highly positive relationship to the yields of dry matter, the yields of protein also are positively related to this factor.

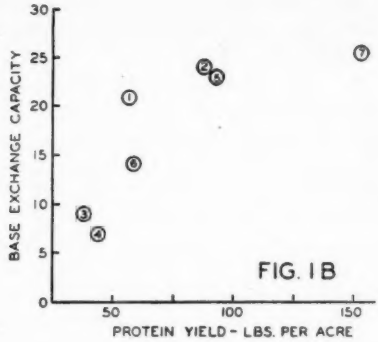
The degree of base saturation may be regarded indirectly as the ratio of the total amount of replaceable bases to nitrogenous organic matter. Figure 3A shows that the leaves containing the higher percentage of protein are from soils having a low degree of base saturation, a fact which indicates that the nitrogen in the organic matter responsible for the base-exchange capacity is of relatively greater importance than the replaceable bases. The yield of dry matter has been shown by Wynd and Noggle (1945) to be but little affected by the degree of base saturation, and therefore the data (Fig. 3B) show a smaller effect on the yields of protein than would be expected from its effect on the percentage of protein.

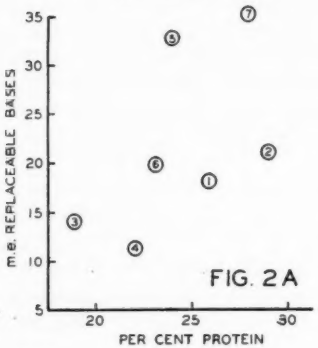
Figures 4A and 4B show that variations in pH bear but little relationship to the percentage of protein or to the yield. This was found to be true also, Wynd and Noggle (1945), for the yield in dry matter; the yield of protein consequently bears no relationship to the pH of the soil.

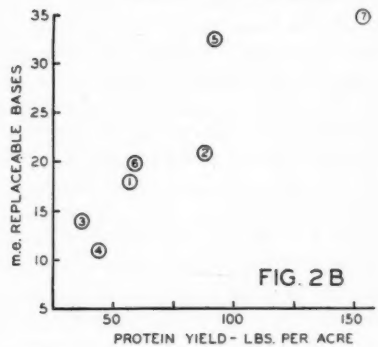
The replaceable calcium appears to be positively related to the protein content of the oat leaves. Calcium comprises such a large proportion of the total replaceable bases that Fig. 5A resembles Fig. 2A, and the discussion of the significance of the total replaceable bases applies equally well to the calcium component. Calcium has a very great effect on the yield of dry matter (Wynd and Noggle, 1945) as it also accompanies an increased percentage of protein. The yield of protein in pounds per acre, therefore, is related to a very high degree to the amount of replaceable calcium in the soil.

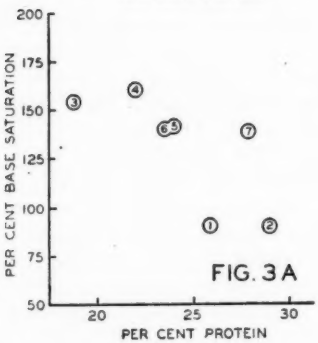
When the replaceable calcium is calculated as a ratio to the amount of total replaceable bases, a strongly negative relationship appears; Fig. 6A indicates that this ratio is of particular importance. This negative relationship probably is not due to any inherent significance of the ratio of calcium to the total positive ions but to the fact that the favorable influence of calcium is less than that of all positive ions. The same situation











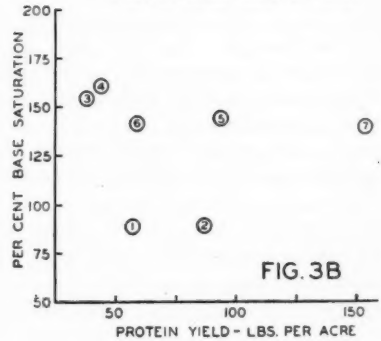


FIG. 1A. Relationship between magnitude of base-exchange capacity of soil and percentage of protein in leaves of oats. FIG. 1B. Relationship between magnitude of base-exchange capacity of soil and yield of protein, in pounds per acre.

FIG. 2A. Relationship between amount of replaceable bases in soil and percentage of protein in leaves of oats. FIG. 2B. Relationship between amount of replaceable bases in soil and yield of protein, in pounds per acre.

FIG. 3A. Relationship between degree of base saturation of soil and percentage of protein in leaves of oats. FIG. 3B. Relationship between degree of base saturation of soil and yield of protein, in pounds per acre.

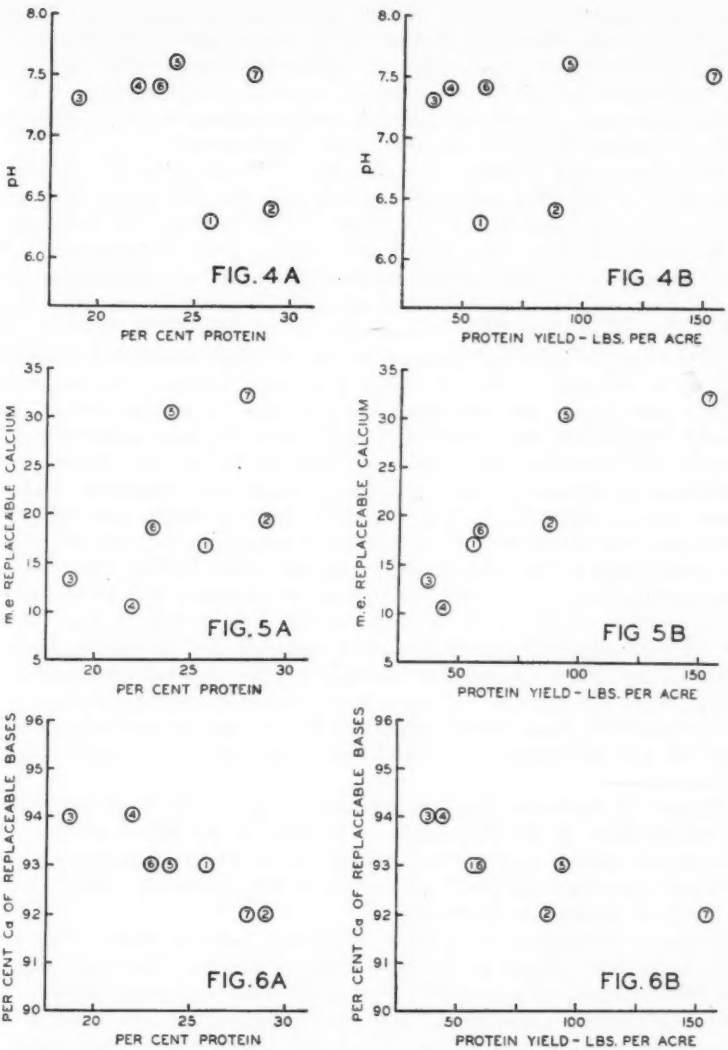


FIG. 4A. Relationship between pH of soil and percentage of protein in leaves of oats. FIG. 4B. Relationship between pH of soil and yield of protein, in pounds per acre.

FIG. 5A. Relationship between amount of replaceable calcium in soil and percentage of protein in leaves of oats. FIG. 5B. Relationship between amount of replaceable calcium in soil and yield of protein, in pounds per acre.

FIG. 6A. Relationship of magnitude of ratio of replaceable calcium to total replaceable bases in soil and percentage of protein in leaves of oats. FIG. 6B. Relationship of magnitude of ratio of replaceable calcium to total replaceable bases in soil and yield of protein, in pounds per acre.

occurs in respect to the yield in dry matter and therefore the yield in protein is closely related to changes in the magnitude of this ratio.

The positive relationship between replaceable magnesium in the soil and the protein content of oat leaves is especially evident (Fig. 7A). A similar relationship to the yield of dry matter causes the yield of protein also to be closely related to the amount of this nutrient.

The relationship between the magnitude of the ratio of replaceable magnesium to the total replaceable bases and the percentage of protein is very different from that to the yield of dry matter. The relationship to the yield was irregular (Wynd and Noggle, 1945) but generally low ratios accompanied high yields because the effect of magnesium itself was less than the combined effect of all positive ions. On the other hand, high ratios accompany high percentages of protein in the leaves. This suggests that the effect of magnesium, or of some component associated with it, is very great since it exerts a greater influence on the protein content than do all the remaining positive ions. A comparison of Figs. 8A and 12A reveals that Points 1, 2, and 7 have the same relative position in both, and therefore the location of these points in both figures could depend on the nitrogen content of the soils which they represent. Further, Points 3 to 6, inclusive, in Fig. 8A show that increasing percentages of protein may be obtained with no significant change in the ratio of replaceable magnesium to the total bases in the soil, while in Fig. 12A the locations of these points are clearly related to the nitrogen content of the soil. The very great similarity in the distribution of the points in Fig. 7A and 11A to their distribution in Fig. 12A suggests the possibility that, in each case, the relative amount of nitrogen in the soil is the most significant factor. Since the influence of variations in the ratio of magnesium to the total replaceable bases affects the yield of dry matter inversely to their effect on the percentage of protein, the total yield of protein remains unchanged.

Figure 9A indicates that the replaceable potassium bears no discernible relationship to the percentage of protein in the leaves of oats. The yield of dry matter is positively related to the higher amounts of potassium and therefore the yield of protein is also positively related to the amounts of replaceable potassium.

Ratios of potassium to the total replaceable bases is shown (Fig. 10A) to be negatively related to the percentage of protein. This fact suggests that the combined effects of the other positive ions is much greater than that of potassium. A similar result was reported for the effect of this ratio on the yield of dry matter, Wynd and Noggle (1945), and therefore the data presented in Fig. 10B show a strongly negative relationship between the magnitude of this ratio and the total yield of protein.

Figure 11A indicates that the amounts of nitrogen in the soil are closely related to the percentage of protein in the leaves of the crop. It has already been suggested in the foregoing discussion that the base-exchange capacity, amount of total replaceable bases, amount of individual replaceable bases, and loss on ignition are all inherently related to the amount of nitrogen in the soil. Each of this group of factors, therefore, appears to bear a positive relationship to the percentage of protein in the

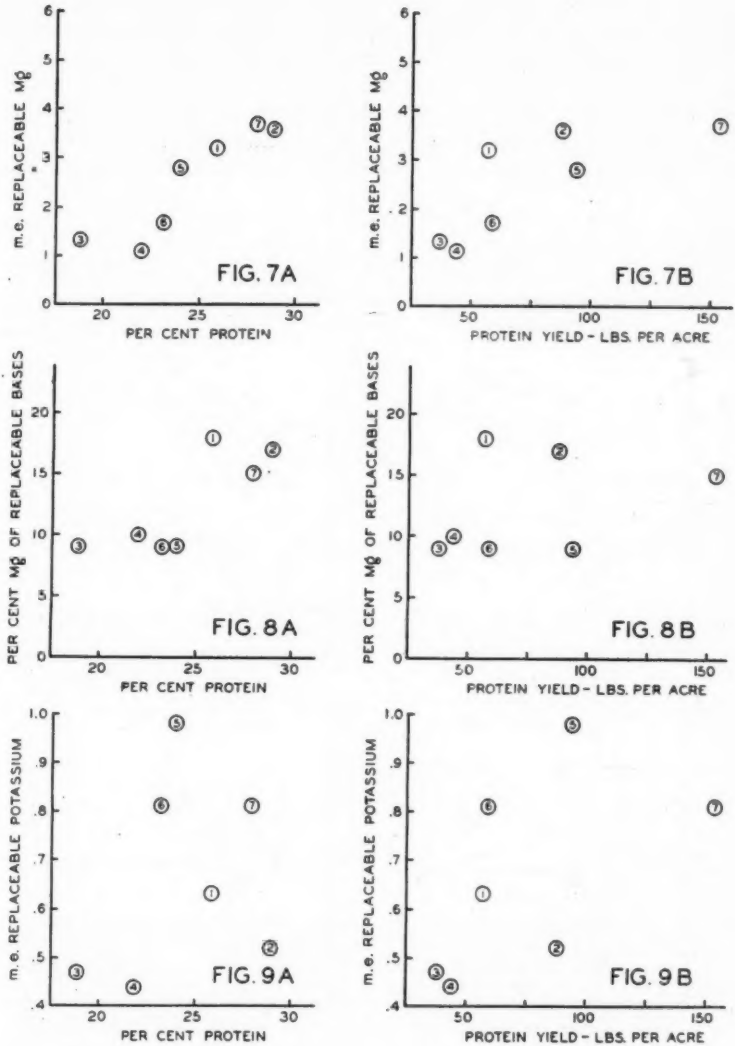
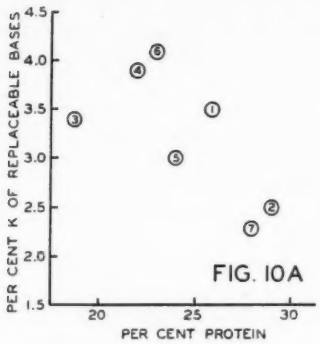
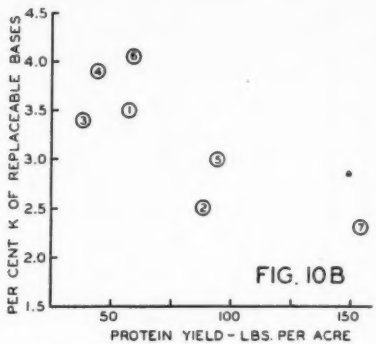


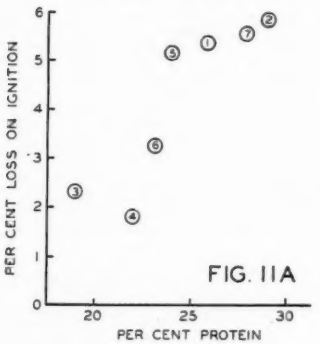
FIG. 7A. Relationship between amount of replaceable magnesium in soil and percentage of protein in leaves of oats. FIG. 7B. Relationship between amount of replaceable magnesium in soil and yield of protein, in pounds per acre.

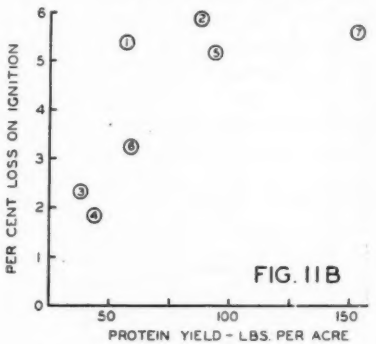
FIG. 8A. Relationship between magnitude of ratio of replaceable magnesium to total replaceable bases in soil and percentage of protein in leaves of oats. FIG. 8B. Relationship between magnitude of ratio of replaceable magnesium to total replaceable bases in soil and yield of protein, in pounds per acre.

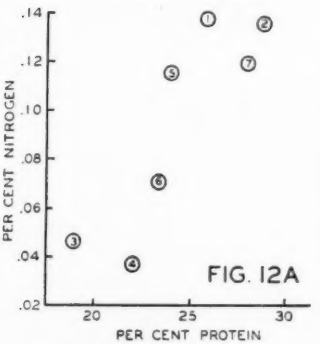
FIG. 9A. Relationship between amount of replaceable potassium in soil and percentage of protein in leaves of oats. Fig. 9B. Relationship between amount of replaceable potassium in soil and yield of protein, in pounds per acre.











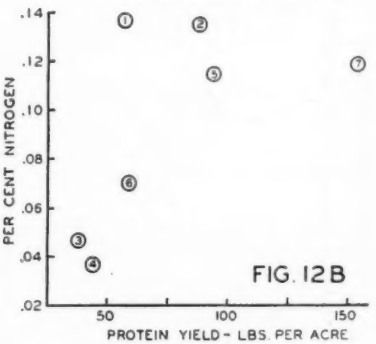


FIG. 10A. Relationship between magnitude of ratio of replaceable potassium to total replaceable bases in soil and percentage of protein in leaves of oats. FIG. 10B. Relationship between magnitude of ratio of replaceable potassium to total replaceable bases in soil and yield of protein, in pounds per acre.

FIG. 11A. Relationship between percentage of soil loss on ignition and percentage of protein in leaves of oats. FIG. 11B. Relationship between percentage of soil loss on ignition and yield of protein, in pounds per acre.

FIG. 12A. Relationship between percentage of nitrogen in soil and percentage of protein in leaves of oats. FIG. 12B. Relationship between percentage of nitrogen in soil and yield of protein, in pounds per acre.

crop. It is important to note that the percentage of protein in the crop is positively related to amount of nitrogen in the soil even when variations in the nitrogen content are not accompanied by similar variations in other members of this group of soil properties.

The amount of phosphorus in the soil that is soluble in weak acid has no observable relationship to the percentage of protein in the leaves. Since there also is no effect on the yield of dry matter, the yield of protein is unaffected.

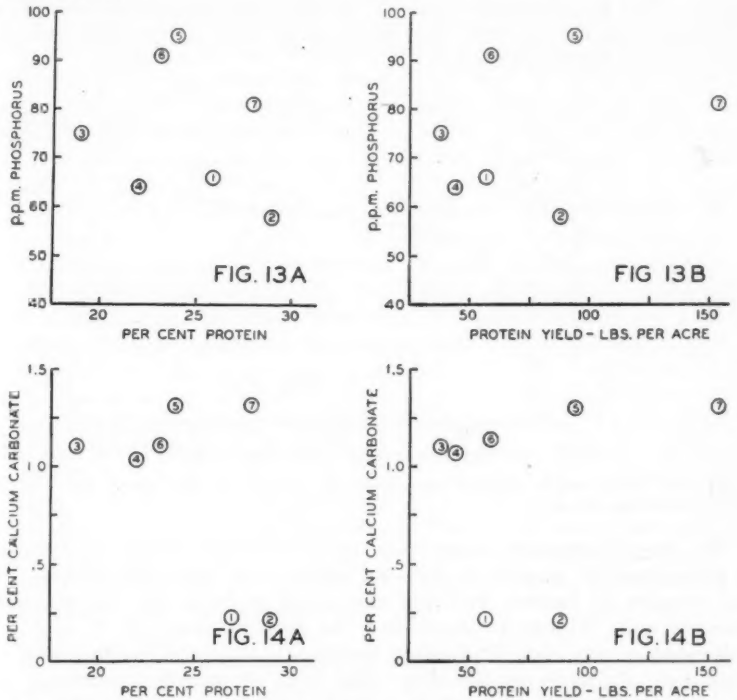


FIG. 13A. Relationship between amount of acid-soluble phosphorus in soil and percentage of protein in leaves of oats. FIG. 13B. Relationship between amount of acid-soluble phosphorus in soil and yield of protein, in pounds per acre.

FIG. 14A. Relationship between amount of calcium carbonate in soil and percentage of protein in leaves of oats. FIG. 14B. Relationship between amount of calcium carbonate in soil and yield of protein, in pounds per acre.

The amount of calcium carbonate in the calcareous soils appears to be slightly related to the percentage of protein in the crop. This is probably due to the larger amount of replaceable bases and nitrogen which are associated with the larger amounts of carbonate. This interpretation is borne out by the fact that Fields 2A and 2B (Points 1 and 2) are very different from the remaining soils in their effect on the amount of protein in the leaves when they are compared on the basis of their carbonate content, yet these soils fall well into line if they are compared on the basis of their nitrogen content or of their replaceable bases.

The data which have been presented suggest that there is usually a positive relationship between the percentage of protein in the tissue and the total yield of protein, in pounds per acre. This positive relationship would be expected if it is assumed that soil factors represented by the complex of factors represented by the organic matter, nitrogen, base-exchange capacity, and total replaceable bases were all varying concomitantly. The organic matter contains the major part of the nitrogen in the soil, and it is also the major component responsible for the base-exchange capacity. The base-exchange capacity, in turn, determines the amount of replaceable bases in the soil, but this is true only if the base-exchange capacity is saturated with bases.

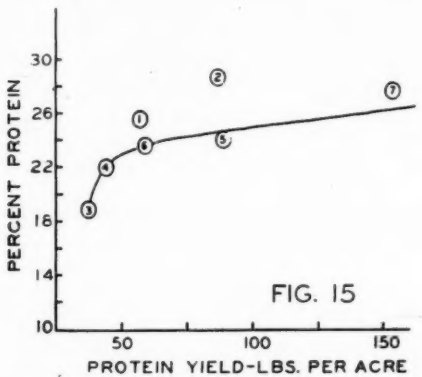


FIG. 15. Relationship between percentage of protein in oat leaves and yield of protein, in pounds per acre.

The above statement means that the production of dry matter and the percentage of protein in the dry matter, are both governed by the same complex of factors, but that this could be true only for the base-saturated soils. Figure 15 shows that the data numbered 3, 4, 5, 6, and 7 represent a very close correlation between the yield of protein and the percentage of protein in the crop. The yield of protein, of course, is a reflection of the production of dry matter and the percentage of protein. These data are all from the soils which are saturated with bases.

Data numbered 1 and 2, on the other hand, are erratically located in the figure. The yields of protein from these fields should have been much larger in view of the high percentage of protein. These soils were not saturated with bases, hence the theory presented above relative to the base-saturated soils would not be expected to apply. In this latter instance, it is apparent that the production of dry matter has been limited by some factor not associated with the percentage of protein.

SUMMARY

The chemical properties of a group of seven soils in the vicinity of Midland, Douglas County, Kansas, were compared on the basis of their effects on the percentage of protein and on the total yield of protein in oat leaves harvested at the jointing stage.

The most important factors associated with the percentage of protein in the tissue were the base-exchange capacity, loss on ignition, and nitrogen. The individual effects of these factors cannot be determined because these factors vary quantitatively with each other in the soils studied. The most important of these is probably nitrogen, since variations in the protein concentration in the crop parallel more consistently variations in the percentage of nitrogen in the soil than the other factors in this group.

The most important factors governing the yield of protein, in pounds per acre, were the base-exchange capacity, total replaceable bases, replaceable calcium, and the percentage of loss on ignition. The most important of these is probably total replaceable bases since the yield of dry matter is dominated by the replaceable bases. The yield of protein is governed by the same factors that determine the yield of dry matter. Replaceable potassium and weak, acid-soluble phosphorus, within the limits of the values studied, appear to bear no relationship to protein accumulation in oat leaves.

Soil factors associated with the higher percentages of protein in the leaves usually, but not always, are also associated with high yields of dry matter and of protein. On base-saturated soils, the growth and the percentage of protein is governed by the organic matter-nitrogen-replaceable bases complex. On the unsaturated soils, the production of dry matter is governed by some factor not associated with the percentage of protein.

Satisfactory soils in the vicinity of Midland for the production of protein in immature oat leaves are characterized by chemical properties having at least the following magnitudes: base-exchange capacity 15 milliequivalents per 100 grams, replaceable bases 20 milliequivalents, replaceable calcium 16 milliequivalents, replaceable magnesium two milliequivalents, nitrogen 0.07 per cent, and loss on ignition three per cent. Soils in this area having a base-exchange capacity less than 10 milliequivalents per 100 grams, replaceable bases less than 15 milliequivalents, and nitrogen less than 0.07 per cent are unsatisfactory for the production of protein in oat leaves harvested at the jointing stage.

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SPECIFIC HEATS OF DEHYDRATED VEGETABLES AND EGG POWDER

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In the course of an investigation of heat transfer through dehydrated vegetables and egg powder, values of the mean specific heats of these materials between room and dehydrator temperatures were required. A survey of the literature revealed that comparatively few measurements of the heat capacities of foodstuffs are on record, and none were found for these dehydrated products which have recently become so important. We have therefore measured the mean specific heats between 27 and 66°C. (80.6 and 150.8°F.) of seven dehydrated vegetables and egg powder. We have also determined how the mean specific heats of three of these materials change with moisture content.

EXPERIMENTAL PROCEDURE

The mean specific heat between 27 and 66°C. was determined by the method of mixtures. The calorimeter consisted of a cylindrical Dewar flask, 85 mm. inside diameter by 260 mm. deep, containing 1,150 ml. of mineral oil as bath liquid. It was provided with a cylindrical, sheet-copper lining to which were soldered a stirrer tube and a support for the sample container. The sample (in ground form) was contained in a cylindrical copper cup of 30 ml. capacity and provided with six radial vanes, a gasket, and a screw top. The top of the Dewar flask was covered by a copper shield dipping into a surrounding constant-temperature bath at 65.95 ± 0.05°C. and provided with openings for stirrer shaft, thermocouple, and introduction of the sample. The sample container was brought to 27°C. by being suspended in an air space within a water bath at 26.95 ± 0.03°C. It was rapidly transferred to the calorimeter bath in a paper shield to minimize heat exchange with the air of the room during the process. The temperature change in the calorimeter bath was measured to within 0.001°C. by means of a 20-junction, copper-constantan thermocouple and precision potentiometer. The apparatus was calibrated and the calibration was periodically checked by measurements with the sample cup empty and containing water. Measurements on several solid substances of known heat capacity (KCl, $NaNO_3$, C_6H_5COOH) gave values which agree within two per cent with those recorded in the International Critical Tables.

A few measurements of mean specific heats between 0 and 27°C. (32 and 80.6°F.) were made with the calorimeter in the 27°C. bath. The sample was brought to 0°C. by placing the sample container within a small, water-tight, screw-top glass jar which was immersed in an ice bath. The jar served both as an air bath at 0°C. and as a temperature shield during transfer of the sample to the calorimeter.

[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

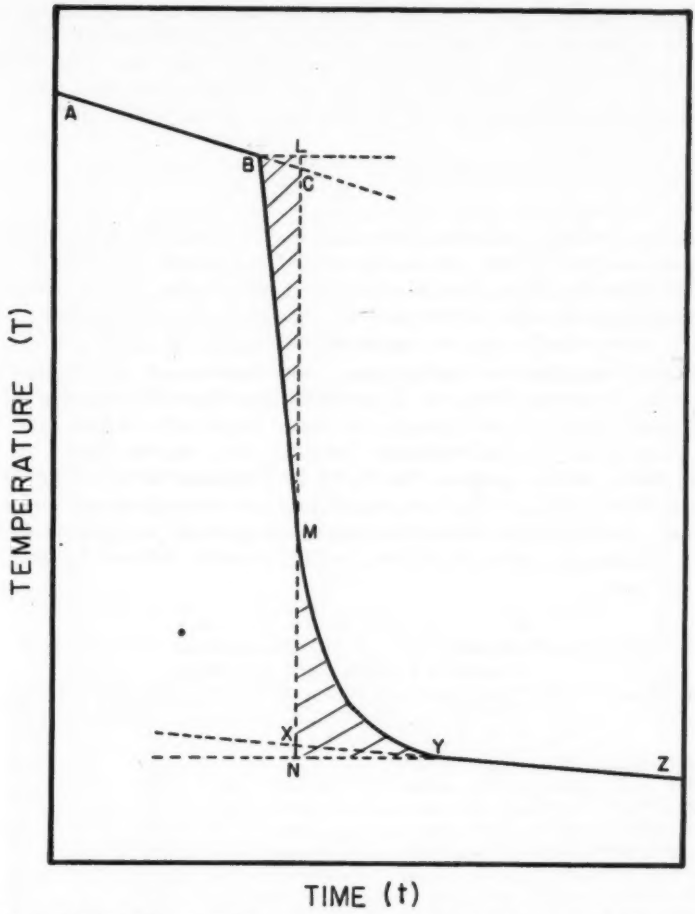

FIG. 1. Method of correcting observed temperature change of calorimeter bath for thermal leakage effects. The time interval from Point A to Point Z is usually about 40 minutes. The temperature change from Point B to Point Y is of the order of one degree Centigrade.

Considerable care must be exercised to obtain reliable specific heat data by the method of mixtures with materials of low heat capacity per unit volume. The heat capacity of our sample container (7.15 cal./°C.) was in many cases as large as that of the sample. It was found important to make adequate corrections for thermal leakage of the calorimeter during the course of an experiment. This was done by the graphical method of Dickinson (1914) adapted to the method of mixtures as illustrated (Fig. 1). The solid line ABMYZ represents the observed temperature readings of the calorimeter bath plotted as a function of time in a typical experiment. It consists of a fore period (AB), a rapid drop after the sample is intro-

duced, and an after period (YZ) which is considered as beginning when the rate of change of temperature has again become essentially constant. The vertical line LMN is constructed at such a time that area BLM is equal to area MNY, where BL and NY are parallel to the time axis. The corrected temperature change of the calorimeter is then given by

$$\triangle T=(T_X-T_C)-\frac{K''}{K'+K''}(T_L-T_C) \qquad (1)$$

where the subscripts indicate points in Fig. 1, and K′, K″ are respectively the heat capacities of the calorimeter and of the charge introduced.[2] Care was taken to minimize the effects of temperature lag of the sample by arranging that the rate of temperature change in the after period (YZ of Fig. 1) be practically zero, as recommended by Jessup (1942).

Moisture contents of the samples were determined in triplicate by drying in a vacuum oven at a pressure less than 10 mm. Hg at the temperature and for the number of hours listed here:[3] eggs 100°C., 5 hr.; beets 70°C., 24 hr.; cabbage 70°C., 6 hr.; carrots 70°C., 24 hr.; onions 70°C., 6 hr.; potatoes 70°C., 44 hr.; spinach 70°C., 6 hr.; sweet potatoes 70°C., 24 hr. The samples of beets, carrots, potatoes, and sweet potatoes used in these determinations were ground to pass a 40-mesh screen. Duplicate moisture determinations usually differed by less than 0.05 per cent.

TABLE 1

Mean Specific Heats Between 27 and 66°C.(80.6 and 150.8°F.) of Dehydrated Vegetables and Egg Powder

Material	Water	Specific heat
	pct.	*cal./g·°C.*
Beets, diced	4.4	.48
Cabbage, leaf	5.4	.52
Carrots, diced	4.4	.50
Carrots, flakes	6.0	.52
Eggs, spray-dried	3.0	.44
Eggs, spray-dried	4.2	.45
Onions, flakes	3.3	.47
Potatoes, diced	6.1	.41
Potatoes, strips	8.0	.46
Spinach, leaf	5.9	.43
Sweet potatoes, diced	7.6	.49

EXPERIMENTAL RESULTS

The mean specific heats between 27 and 66°C. of several different dehydrated vegetables and of egg powder are listed (Table 1). Each entry in the table represents a sample of different origin. The original form of the material is indicated in the first column. Each mean specific heat

[2] This equation is based on Newton's law of cooling. Since $K' >> K''$ and $(T_L\text{-}T_C)$ is small, the last term of (1) may be omitted for all but very precise measurements.

[3] The choice of these drying procedures is based on preliminary results obtained by Dr. B. Makower of this laboratory for carrots, eggs, cabbage, potatoes, and onions in an investigation of the determination of moisture in dehydrated vegetables and eggs.

listed is the average of at least two determinations. The experimental error is estimated to be not more than 0.01 cal./g-°C.

Data on the variation with moisture content of the mean specific heats of dehydrated carrots, eggs, and potatoes are summarized (Table 2). None of the materials used for this study is represented in Table 1. The samples of different moisture levels of each dehydrated product were prepared from portions of the same original material. The egg powder was a commercial, spray-dried product containing, originally, approximately three per cent moisture. The dehydrated carrots (Red Cored Chantenay) and potatoes (Idaho Russet) were dried in the tunnel dehydrator in the pilot plant of this laboratory in the form of ⅜-inch cubes and had initial moisture levels of 7.8 and 8.1 per cent, respectively. They were first ground to pass a 40-mesh screen. The samples of lowest moisture level (Table 2) were prepared by drying in the vacuum oven by the procedure used in determining moisture content. The other moisture levels were obtained by reducing the water content to a low value in a high vacuum at room temperature and subsequently adding *in vacuo* a measured quantity of water as vapor. The final moisture contents were determined by the vacuum-oven method. The samples, once prepared, were kept at room temperature in iodine flasks with mercury seals. In most cases at least three days were allowed for attainment of uniform moisture distribution in a sample after its moisture content had been adjusted.

TABLE 2

Variation With Moisture Content of Mean Specific Heat Between 27 and 66°C.(80.6 and 150.8°F.)

Carrots		Eggs		Potatoes	
Water	Specific heat	Water	Specific heat	Water	Specific heat
pct.	*cal./g-°C.*	*pct.*	*cal./g-°C.*	*pct.*	*cal./g-°C.*
0.15	.344	0.15	.403	0.15	.311
0.95	.361	0.45	.404	0.75	.317
1.85	.415	0.55	.402	2.15	.330
2.10	.413	0.70	.399	4.15	.352
3.50	.474	1.30	.415	7.15	.389
4.25	.489	2.80	.436	8.05	.409
6.80	.495	3.20	.431	10.85	.443
9.30	.513	5.40	.465	15.95	.514
14.50	.543	5.65	.476	.....	.....
.....	.....	8.35	.491	.....	.....
.....	.....	8.50	.497	.....	.....
.....	.....	14.50	.544	.....	.....

Most of the mean specific heats listed (Table 2) are averages of three or four determinations on two or more portions of the same sample. Two successive determinations made on the first portion usually agreed within experimental error, indicating that no irreversible changes affecting the heat capacity occurred during a determination. Measurements on a second portion were made in most cases more than a week later. The agreement of these later measurements with the earlier ones shows that uniform moisture distribution had probably been attained. The mean deviation from the specific heat values listed (Table 2) is 0.004 cal./g-°C. for 29

individual determinations on carrots. The same is true for potatoes. Thirteen determinations on eggs at moisture levels below one per cent show a mean deviation of 0.012 from the values listed, whereas 22 determinations at higher moisture levels show a mean deviation of 0.004 unit. The moisture content of each sample (Table 2) is the average of the moisture levels of the sample before and after it had been used for specific heat determinations. The difference between the "before" and "after" moisture levels was usually less than 0.1 per cent, the sign and magnitude depending on the moisture level of the sample. This change probably occurred during transfer of the sample from one container to another.

Measurements of mean specific heats between 0 and 27°C. were made on six of the samples of Table 2 and on the sample of onions of Table 1. These data and the mean temperature coefficients of specific heat calculated for these samples are given (Table 3).

TABLE 3

Mean Specific Heats Between 0 and 27°C.(32 and 80.6°F.) and Mean Temperature Coefficients of Specific Heat Between 0 and 66°C.(32 and 150.8°F.)

Material	Water	Specific heat	Temperature coefficient
	pct.	*cal./g-°C.*	*cal./g-°C.*
Carrots	0.15	.280	.0019
Carrots	3.50	.327	.0044
Eggs	0.55	.375	.0008
Eggs	14.50	.530	.0004
Onions	3.30	.315	.0048
Potatoes	0.75	.288	.0009
Potatoes	8.05	.335	.0021

DISCUSSION

The moisture contents of various commercially dehydrated vegetables lie in the range three to eight per cent. The mean specific heats between 27 and 66°C. of vegetables in this moisture-level region are seen (Tables 1 and 2) to lie between 0.35 and 0.55 cal./g-°C. For the temperature interval from 0 to 27°C. these limits are estimated from Table 3 to be 0.30 and 0.45 cal./g-°C. The mean specific heat of whole-egg powder is found to be about 0.40 at one per cent moisture and 0.44 at four per cent moisture (Table 2), and to change very slowly with temperature (Table 3).

Comparison of the data for the various samples of eggs, carrots, and potatoes of Table 1 with those of similar moisture content but of different origin in Table 2 shows agreement within 0.01 unit for eggs, differences of 0.01 and 0.02 for carrots, and differences of 0.03 and 0.06 for potatoes. Sufficient data are not available to indicate how great may be the variations in specific heat encountered with samples of a vegetable having the same moisture content but differing with regard to variety, cultural conditions, and processing and storage history. For this reason the uncertainty of the values of Table 1 for general application may be several times as large as the experimental error of 0.01 unit stated above.

Carrots, eggs, and potatoes were chosen for a study of variation of specific heat with moisture content because of the major differences in

their compositions. On the dry basis, carrots are nearly two-thirds sugar, potatoes about two-thirds starch, and eggs approximately half protein and half fat [Chatfield and Adams (1940)]. The heat capacity (C_n) of that quantity of material which contains one gram of dry solid has been calculated from the data (Table 2) and is shown graphically (Fig. 2) as a

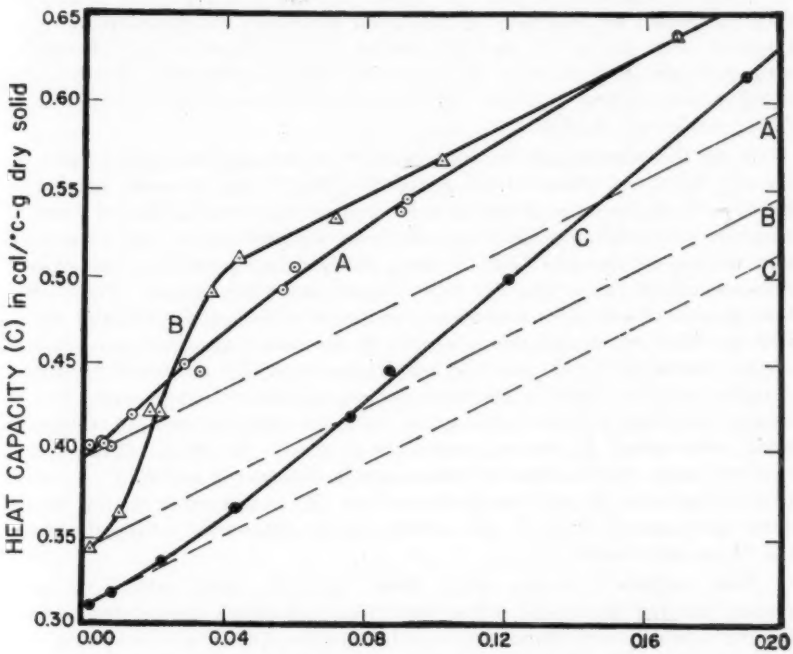

FIG. 2. Heat capacities of dehydrated eggs (A, open circles), carrots (B, triangles), and potatoes (C, filled circles) as a function of moisture content. The solid curves represent experimental values and the dashed lines represent the sums of the heat capacities of the equivalent amounts of dry solid and water. The heat capacity here refers to that amount of material which contains one gram of dry solid.

function of the moisture content (n) expressed in grams of water per gram of dry solid. It is readily seen from the figure that these materials differ markedly in their dependence of heat capacity on moisture content.[4]

If the heat capacity of each sample were the sum of the separate heat capacities of the equivalent quantities of dry solid and water, the heat

[4] The following relations are given for convenient use of Fig. 2 in terms of specific heat: $C_n=(1+n)S$, where S is the specific heat at 100 w per cent water and $w=\frac{n}{1+n}$.

Throughout this paper the reader should bear in mind the distinction between heat capacity and specific heat. As used here, heat capacity may refer to any arbitrary amount of material chosen for discussion, whereas specific heat is the heat capacity per gram of material.

capacity would be a linear function of the moisture content in accordance with the equation

$$C_{na}=d+n \qquad (2)$$

Here C_{na} is the calculated heat capacity of that amount of material which contains one gram of dry solid, d is the specific heat of dry solid, and n is the number of grams of water per gram of dry solid. The heat capacities (C_{na}) expected on the basis of this total additivity relationship are represented (Fig. 2) by the parallel dashed straight lines which coincide at zero moisture content with the experimentally determined curves. The experimental values (C_n) are seen to be higher than those expected from (2) in each case studied.

If, on the other hand, the heat capacity is not additive with respect to the dry solid and water (total additivity, Eq. 2) but at some particular water content becomes additive with respect to the addition of further moisture (differential additivity), the heat capacity above that water content will be represented (Fig. 2) by a straight line parallel to the dashed lines calculated from (2). The experimental curve for carrots (Fig. 2) has been drawn as a straight line corresponding to differential additivity above four per cent water, and this is seen to fit the data reasonably well. It also appears from the figure that the heat capacity of eggs may well be differentially additive above a moisture content of about 15 per cent. In the case of potatoes, however, the figure indicates that differential additivity must occur above 16 per cent moisture if at all. Freeman (1942) found that the mean specific heat of potato starch between 0 and 40°C. at moisture levels below 23 per cent deviates from (2) in a manner similar to the curve for potatoes (Fig. 2) but corresponds to differential additivity above the 23-per cent level.

Heat capacities larger than those expected from additivity with respect to dry solid and water have been observed for at least three hydrophilic colloidal materials, namely cellulose [Shipley, Campbell, and Maass (1939)], gelatin [Hampton and Mennie (1934) and Horn and Mennie (1935)], and potato starch [Freeman (1942)]. On the other hand, crystalline hydrates show negative deviations from (2). In general it is expected that the more firmly water is held, the lower will be its contribution to the heat capacity. In order to account for these positive deviations from total additivity, the investigators mentioned above have suggested that the heat measured experimentally includes not only the heat required to change the temperature of the materials, but also heat that is due to a shift in the water equilibrium within the material as the temperature is changed. Since the heat that is due to desorption or adsorption from the gaseous state is not appreciable in a closed system, such as that used in these experiments, such a postulated shift in equilibrium must involve essentially a change in the energy of binding of the water present. This binding is probably largely through hydrogen bonds [e.g., Babbitt (1942) and Sponsler, Bath, and Ellis (1940)] so that such a shift in equilibrium would involve breaking of hydrogen bonds. However, it is just such a process which is believed to be responsible for the high specific heat of water, our principal reference standard. It seems doubtful, therefore, if

there is much to be gained by trying to distinguish between heat required to change the temperature and heat effects owing to a shift of equilibrium in systems such as these.

The investigators who have advanced the above explanation of the high observed heat capacities of hydrophilic colloid-water systems have all assumed that the contribution of the nonaqueous portion to the total heat capacity is the same as the heat capacity of the dry solid. Although this assumption seems reasonable for systems such as sand and water in which sorbed water is limited to the external surface of the particles [Freeman (1942)], we believe it should not be applied to systems in which sorption of water involves a penetration into the interior of the particles. The penetration of moisture into a dry hydrophilic colloid may be thought of as decreasing the rigidity of the interior structure, thus permitting greater freedom of oscillation (and perhaps rotation) of the units of structure involved with a consequent increase in the contribution of the nonaqueous material to the heat capacity of the system. On the other hand, the more firmly the colloid binds the water present, the lower will be the contribution of this water to the heat capacity of the system. The relative magnitude of these two effects determines whether or not the heat capacity is greater or less than that expected on the basis of total additivity. The available data on hydrophilic colloids indicate that the former of these two effects probably predominates in most cases.[5]

The use of the variation of specific heat with moisture content as another means of determining the "bound water" content of lyophilic systems has recently been suggested by Freeman (1942). He found that the maximum "bound water" content of starch-water suspensions as determined by three different methods was practically the same as the moisture content above which the heat capacity shows differential additivity. Hampton and Mennie (1934) and Horn and Mennie (1935) also interpreted the corresponding moisture content in the gelatin-water system in the same way. From this point of view one would interpret the data shown (Fig. 2) as indicating a maximum "bound water" content at 27°C. of four per cent for (dehydrated) carrots and approximately 15 per cent for (spray-dried) whole eggs.

Let us examine the interpretation of Fig. 2 from the thermodynamic point of view. We shall represent the uptake of n grams of liquid water (nW) by one gram of dry solid (D) by the equation

$$D + nW = D_n + H_n \qquad (3)$$

where D_n represents one gram of dry solid combined with n grams of water and H_n is the heat evolved in the process, which we shall term the heat of hydration. We assume here that D_n is a homogeneous product, the nature of which is independent of how the process (3) is carried out. The rate of change in H_n with temperature is then given by [e.g., Lewis and Randall (1923)]

$$\frac{dH_n}{dT} = -(C_n - C_{na}) \qquad (4)$$

[5] The data of Kotukov (1936) on the heat capacity of gum arabic sols above 80 per cent water content indicate that this system may show negative deviations from total additivity.

where C_n-C_{na} is the difference between the observed heat capacity of D_n and that calculated by (2), in other words the difference between the experimental curve and the corresponding dashed line of Fig. 2. Thus from Fig. 2 it is found that the heat of hydration for two-per cent-moisture carrots decreases on the average by 0.054 calorie per degree centigrade per gram of dry material in the temperature range studied.

The differential heat of wetting ($\overline{H}_n$) is the heat evolved when one gram of liquid water is taken up by a large quantity of material of moisture content n. The rate of change of $\overline{H}_n$ with temperature is likewise given by an equation similar to (4), namely

$$\frac{d\overline{H}_n}{dT} = -(\overline{C}_n - \overline{C}_{na}) \qquad (5)$$

where $(\overline{C}_n - \overline{C}_{na})$ is the difference between the slope of the experimental C_n *vs.* n curve of Fig. 2 and that of the straight-line relationship of (2). Thus from Fig. 2 it is found that the differential heat of wetting of two-per cent-moisture carrots decreases on the average by approximately 4.4 calorie per degree centigrade per gram of water in going from 27 to 66°C.

The definition of maximum "bound water" content in hydrophilic colloidal systems as the water content above which the heat capacity shows differential additivity ($\overline{C}_n = \overline{C}_{na}$) is seen from (5) to be thermodynamically equivalent to the moisture level above which the differential heat of wetting is independent of temperature.

It has been found calorimetrically for cellulose, Argue and Maass (1935); rice, wheat, and potato starches, Winkler and Geddes (1931); and several other hydrophilic colloids, Katz (1917), that the differential heat of wetting decreases as n increases. Furthermore, Fig. 2 in the light of (5), indicates that at moisture contents below differential additivity, the differential heat of wetting decreases with increasing temperature. Combining these two results, one finds that the higher the temperature the lower will be the moisture content at which the differential heat of wetting approaches zero. A very small temperature coefficient of the differential heat of wetting above a given moisture level is therefore probably equivalent to a small value of the differential heat of wetting itself. It also follows that the above definition of maximum "bound water" content is probably equivalent for most hydrophilic colloidal systems to the moisture level above which the differential heat of wetting is small at the lower temperature used in measuring the heat capacity.

The specific heats of foodstuffs have frequently been estimated on the basis of their moisture contents in the absence of direct experimental data. For example, in Lange's *Handbook of Chemistry* (1941) there is a rather extensive table in which specific heats above the freezing point have been calculated from moisture contents by use of the equation

$$S = a + (1 - a)\,w \qquad (6)$$

Here S is the specific heat, 100 w is the percentage water, and a has the value 0.20 cal./g-°C. in the table referred to above. This is equivalent to

assuming additivity of the heat capacities of the dry solid and water with 0.20 as the specific heat of the dry material. Such estimates cannot be far wrong for fresh foods with a high water content, but they are less reliable the lower the moisture content. We have seen above that the specific heat cannot be adequately described as a linear function of the moisture content over the complete moisture range. Our data further indicate that 0.20 is probably too low a value for the specific heat of completely dry vegetables, even for the range from 0°C. to room temperature [see also Zhadan (1939)]; and that if estimates are made on the basis of (6), a value of a higher than the specific heat of the dry material should be used to compensate for the observed positive deviations from additivity of the heat capacities of dry solid and water. Until additional data are available, we recommend use of (6) with $a = 0.45$ for the estimation of the mean specific heats of vegetables between 25 and 65°C. and with $a = 0.32$ for the range from 0 to 25°C. This latter value of a is that found by Ryutov and Veselova (1939) to be suitable for fresh fruits and vegetables.

Zhadan (1939, 1940) has suggested that specific heats of foodstuffs can be reliably estimated from their proximate compositions if one assumes additivity of the heat capacities of the separate components present. He uses 0.30, 0.35, and 0.40 cal./g-°C. as the specific heats at 0°C. of water-free carbohydrates, proteins, and fats, respectively, with temperature coefficients of 0.0015 cal./g-°C.[2] for carbohydrates and proteins and of 0.005 for fats. Our data indicate, however, that deviations from additivity may be larger than Zhadan's estimates of differences in specific heats of various foods owing to differences in composition on the dry basis. It would seem therefore that until more specific heat data on various foodstuffs are available, estimates based on (6) with suitable values of a are probably as reliable as those calculated from proximate composition data by Zhadan's method.

SUMMARY

The mean specific heats of seven dehydrated vegetables and of egg powder have been measured for the temperature interval 27 and 66°C. (Tables 1 and 2). Measurements between 0 and 27°C. were also made on four of these materials (Table 3).

The heat capacity was found to exceed the sum of the heat capacities of the equivalent quantities of dry solid and water by an amount which varies with moisture content in different ways for dehydrated carrots, eggs, and potatoes. These positive deviations from additivity are discussed with regard to origin, thermodynamic significance, and interpretation in terms of "bound water" content.

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PHYSICAL PROPERTIES OF STARCH FROM POTATOES OF DIFFERENT CULINARY QUALITY

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Many studies have been made of the physical properties of potato starch but few have been related to the culinary quality of potatoes. Those of Sweetman (1936) are almost unique in this respect. She found no correlation between culinary quality of potatoes and either the swelling capacity of the starch therefrom or the viscosity of starch pastes.

This paper reports the results of a two-year study of some physical properties of starch from potatoes of different degrees of mealiness. The following characteristics were studied: distribution of granule size; changes occurring in starch granules when heated in water; consistency and translucency changes in heated starch suspensions; the form of the heating curves of heated starch suspensions; the rigidity and the resistance to penetration of cooled starch pastes. The data were evaluated in terms of the relative mealiness of the potatoes.

EXPERIMENTAL PROCEDURE

Twenty-two lots of potatoes, comprising nine varieties, were examined, 11 from the crop of 1939 and 11 from the crop of 1940 (Table 1).

Starch was separated from the finely comminuted, raw, peeled tubers by washing it through cheesecloth. It was then washed free from potato juice, filtered, and dried at room temperature. Examination of random samples of the various starches showed them to be free of fibrous material.

All of the water used throughout the study was distilled water.

Mealiness: The degree of mealiness of steamed tubers was rated on a scale of 10, zero being very soggy and 10 being very mealy. Ten tubers from each lot of potatoes were judged. Ratings for nine of the 1939 lots were obtained from subjective data collected as part of a co-operative project in progress in the Department of Food and Nutrition of the New York State College of Home Economics. The other samples were rated by two of the authors (A.M.B. and E.G.C.).

Granule Size: Distribution of granule size was determined by hydrometer analysis as described by Bauer (1937).

Loss of Anisotropy: Changes occurring in heated starch granules were observed under a microscope using polarized light. Dilute suspensions were heated by means of a hot stage fitted with an automatic switch permitting control from room temperature up to 85°C.(185°F.). A water suspension of starch was heated rapidly in the hot stage and record was made of the temperature at which isotropic starch granules were first

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TABLE 1

Description of Samples, Temperatures at Which Changes Occurred in Starch Granules in Suspensions When Heated in Water, and Rigidity and Penetration Values for Cooled Starch Pastes

Sample No.	Variety	Where grown	Degree of mealiness[1]	Temperatures				Rigidity	Penetration
				Starch granules		Starch-water suspensions			
				At first loss of anisotropy	At final loss of anisotropy	At break in heating curve	At increased translucency		
1939				°C.	°C.	°C.	°C.	*dynes/100 per sq. cm.*	*gm.*
11	Bliss Triumph (?)	Texas	2.0	60.8	71.8	68.0	70.8	132	108
8	Chippewa	Wayne Co., N.Y.[2]	2.0	51.8	69.1	67.6	68.8	146	136
9	Irish Cobbler	Wayne Co., N.Y.	2.6	61.5	70.9	68.1	70.0	172	152
7	Erlaine	Tompkins Co., N.Y.	4.6	58.6	70.6	66.3	67.2	96	90
4	Chippewa	Tompkins Co., N.Y.	5.0	60.4	70.0	67.6	68.0	103	138
6	Green Mountain	Tompkins Co., N.Y.	5.2	58.7	70.3	66.3	67.5	106	106
3	Pioneer Rural	Tompkins Co., N.Y.	5.4	59.2	68.8	66.5	67.7	74	111
5	Katahdin	Tompkins Co., N.Y.	5.4	58.4	67.9	68.0	68.5	128	118
2	Chippewa	Tompkins Co., N.Y. (McLean)	5.6	61.2	71.2	68.5	69.7	112	106
1	Smooth Rural	Tompkins Co., N.Y. (McLean)	5.6	57.4	66.1	66.3	67.4	102	110
10	Russet Burbank	Idaho	8.5	54.1	63.4	64.0	65.1	67	126
1940									
21	Chippewa	Wayne Co., N.Y.	1.3	59.0	72.3	67.9	68.7	177	128
20	Irish Cobbler	Wayne Co., N.Y.	1.5	57.4	70.6	67.4	68.9	286	130
16	Chippewa	Tompkins Co., N.Y.	1.8	57.4	70.6	66.0	68.0	124	103
18	Erlaine	Tompkins Co., N.Y.	2.5	58.5	72.2	65.7	67.4	104	107
17	Katahdin	Tompkins Co., N.Y.	5.4	57.9	70.7	66.5	67.7	126	106
13	Chippewa	Tompkins Co., N.Y. (McLean)	5.4	56.7	67.9	66.7	67.4	95	123
15	Pioneer Rural	Tompkins Co., N.Y.	6.5	59.3	68.8	66.8	68.0	61	84
14	Katahdin	Tompkins Co., N.Y. (McLean)	6.5	57.2	69.6	65.9	68.2	96	103
12	Smooth Rural	Tompkins Co., N.Y. (McLean)	7.0	56.2	68.8	64.1	65.1	93	105
22	Russet Burbank	Idaho	8.7	54.4	71.1	65.5	66.6	96	111
19	Green Mountain	Tompkins Co., N.Y.	8.7	53.9	68.4	64.9	65.7	114	118

[1] The higher the number the more mealy the potato. [2] The N. Y. samples can be divided into three groups on the basis of soil type and cultural practice: (1) those grown in Tompkins Co., N. Y.; (2) those grown in Tompkins Co., N. Y. (McLean); (3) those grown in Wayne Co., N. Y. Nothing is known of soil type or cultural practice for the remaining three samples.

observed and again when the last signs of anisotropy had just disappeared from the starch grains. A copper-constantan thermocouple in the cell of the hot stage and attached to a Leeds and Northrup potentiometer was used for measuring the temperature of the starch suspensions.

Consistency and Translucency: Sixteen-per cent water suspensions of air-dried starch were used for all other observations on heated starch suspensions and on cooled starch pastes. The ratio of starch to water in potatoes is, on an average, a little less than 1:5; this approximates a 19-per cent water suspension of starch.

Starch-water suspensions were heated in a boiling-water bath either for 20 minutes or until a specified temperature was reached in the center of the paste. Until the mixture thickened, it was kept well stirred by means of a loop stirrer, used with an up and down motion. The thickening was very rapid once it began, being completed in a few seconds. The temperature at which this change took place was noted. Soon after thickening, the paste gradually lost its milky appearance and note was made of the temperature at which the entire paste became translucent.

Heating Curves: For the heating curves, a thermometer graduated in 0.2°C. was supported in the middle of the suspension and temperature readings were made at 10-second intervals.

Penetration and Rigidity: Penetration measurements were made on cooled starch pastes; the weight required to cut through one millimeter of sample was determined. The apparatus for penetration measurements was described by Pfund (1938). It is an instrument designed to measure the weight in grams required to force taut wires a measured distance through a piece of the material being tested.

Rigidity measurements were made on cooled starch pastes in a rigidometer described by Brimhall and Hixon (1939). The instrument is designed to measure the resistance offered by a gel to a torsional force.

RESULTS AND DISCUSSION

Particle-Size Distribution: Six of the 1939 and nine of the 1940 starch samples were examined for particle-size distribution. For all samples a curve of similar form (Fig. 1) was obtained when percentage of granules less than a specified diameter was plotted against diameter of granules. There is no correlation between the mealiness of potatoes and the percentage of granules above 0.03 mm. diameter. However, the coefficient of correlation between mealiness and the percentage of granules below 0.02 mm. diameter is —0.724 for the 1939 and —0.801 for the 1940 samples. The former figure is slightly significant,[2] but the latter is significant, indicating that the more there are of the smaller starch granules in a potato, the less mealy the potato is likely to be.

Losses in Anisotropy and Other Changes Occurring in Starch Granules When Heated in Water: When starch granules in water were heated rapidly and observed under the microscope, a wrinkling appeared around the hilum and as this disappeared, a swelling set in and gradually the

[2] Throughout this discussion correlations are reported as significant if, using Student's "t" test, the odds are 99:1 or greater, and are reported as slightly significant if the odds are at least 19:1.

those grown in Tompkins Co., N. Y.; (2) those grown in Tompkins Co., N. Y. (McLean); (3) those grown in Wayne Co., N. Y. Nothing is known of soil type or cultural practice for the remaining three samples.

starch grain assumed its final swollen, isotropic form. This change began first at about 58°C.(136.4°F.) in a few grains, and as the temperature continued to rise, more grains became swollen and isotropic until eventually all grains were swollen and had lost all signs of anisotropy at about 70°C.(158°F.). Most of the granules underwent this change within a narrow temperature range, about 2 or 3°C. The swollen granules assumed the variety of forms described by Sjostrom (1936). The last granules to lose anisotropy were always small ones, although some small granules would be among the first to undergo this change.

When the temperature of the suspension was held stationary after some of the granules had become swollen, the process of gelatinization was arrested until the temperature was again allowed to rise.

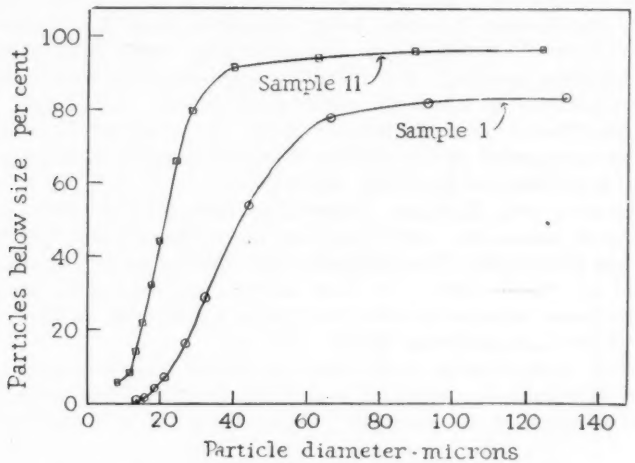

FIG. 1. Particle-size distribution curves of potato starches.

NOTE—All other particle-size distribution curves are similar in form and lie between the two illustrated.

The coefficient of correlation between the degree of mealiness of the steamed tubers and the temperature at first loss of anisotropy for the 1939 samples is —0.618 and for the 1940 samples is —0.673. Both values are slightly significant indicating that the starch from the more mealy potatoes first showed loss of anisotropy at a lower temperature than did the starch from the soggy potatoes.

The data for the 1939 samples showed an inverse relationship between mealiness and the temperature at complete loss of anisotropy but no such relationship was apparent for the 1940 samples. The correlation coefficients were —0.733 (significant) and —0.601 (slightly significant), respectively.

Consistency and Translucency Changes in Heated Starch Suspensions and Form of Heating Curve: When starch suspensions were heated a sudden thickening took place and simultaneously the temperature stopped rising and remained stationary until the paste had become partially translucent. The temperature recorded as the temperature of sudden thick-

ening was also the temperature of the break in the heating curve; the temperature of complete translucency was from 0.4 to 2°C. above this (Table 1). The temperatures at which these changes took place were characteristic for each sample and showed little variation between replicate determinations. The heating curves for all samples were very similar in form, varying mainly in the temperature at which the break took place (Fig. 2).

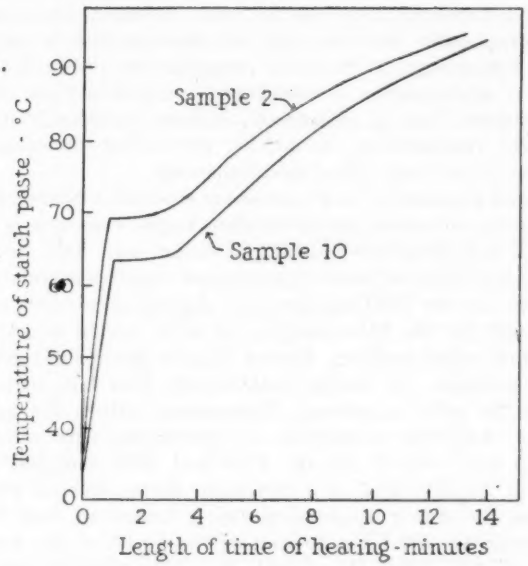

FIG. 2. Representative heating curves of 20-per cent starch-in-water suspensions.
NOTE—Of all the samples examined, the starch from Sample 2 had the highest and from Sample 10 the lowest temperature of break in the heating curve.

The coefficient of correlation between mealiness and the temperature of both the sudden thickening and the break in the heating curve is —0.645 for the 1939 samples and —0.637 for the 1940 samples. Both are slightly significant indicating that these two characteristic changes which are observed during the heating of starch suspensions tended to occur at lower temperatures in the starches from the more mealy potatoes.

The observation of a break in the heating curve of potato-starch suspensions was reported by Nazarov and Nikolajev (1938) who found an endothermic stop at the temperature at which they expected gelatinization would begin. Küntzel and Doehner (1939) studied this thermal effect more precisely and found a sudden fall in temperature soon followed by a gradual rise. The first effect was observed a short time before the decrease in opacity, and this fall in temperature reached its minimum after the suspension had begun to lose its opacity. In general, the results of our experiments are in agreement with the above observations. Furthermore, the heat of the endothermic reaction was not very great; the break in

the heating curve was scarcely perceptible in five-per cent starch-water suspensions.

Whether this endothermic process is due to the heat of rupture of the bonds in the starch grains, as postulated by Nazarov and Nikolajev (1939), or to the "melting" of the crystalline structure within the starch grain, as suggested by Küntzel and Doehner (1939), or to absorption of water prior to, or coincident with, the transition of starch grains, as proposed by Katz and Seiberlich (1938), is an open question. It is evident from the microscopic studies, however, that all starch grains in any sample do not undergo the change at the same temperature. Also, for any starch the so-called "gelatinization temperature"[3] depends upon the criterion used—the complete loss of anisotropy, sudden increase in translucency, maximum light transmission, or sudden thickening—inasmuch as all of these changes do not take place simultaneously.

Rigidity and Resistance to Penetration of Cooled Starch Pastes: The values for rigidity of pastes, obtained when starch suspensions were heated 20 minutes in a boiling-water bath and cooled and held undisturbed at 25°C.(77°F.) for 24 hours, showed significant negative correlation, —0.794, with mealiness for the 1939 samples and slightly significant negative correlation, —0.630, for the 1940 samples. In other words, weight for weight, the starch from soggy potatoes tended to give more rigid gels than that from mealy potatoes. A similar relationship was not apparent in the resistance of the gels to cutting. Penetration values for gels similarly prepared gave very low coefficients of correlation with mealiness, these being —0.225 and —0.349 for the 1939 and 1940 samples, respectively.

A series of rigidity and of penetration measurements were made on eight samples, the starch suspensions being heated to final temperatures ranging from 98°C.(208.4°F.) to that of the break in the heating curve, 64 to 69°C.(147.2 to 156.2°F.), (Table 2). Both rigidity and penetration values increased markedly with decreasing final temperatures. The increase in the rigidity values was fairly regular; the increase in the penetration value was somewhat irregular and was not of the same magnitude as the increase in rigidity. The degree of mealiness of the steamed potatoes appeared unrelated to the magnitude of the increases. The starch gels showed maximum strength when the suspensions were heated till just thickened. This is contrary to the results of Woodruff and Nicoli (1931) who obtained maximum gel strength for potato starch by heating the suspension to a temperature of 90°C.(194°F.) or higher. They used a five-per cent starch-water suspension which may account for the difference.

SUMMARY AND CONCLUSIONS

A study was made of some physical properties of starch extracted from 22 samples of potatoes of different degrees of mealiness.

Using a hydrometer method, for all of the starch samples the distribution of particle size was found to follow a sigmoid curve when the percentage of particles below a specified diameter was plotted against diameter. There appeared to be a negative correlation between the number of starch

[3] A term used by workers to designate the temperature at which certain definite physical changes take place in a suspension of starch in water on heating.

granules below 0.02 mm. in diameter and the degree of mealiness of the cooked potatoes.

Determination was made of the temperature at which the first starch grains of a given sample lost anisotropy and the temperature at which the last signs of anisotropy just disappeared from all starch grains. These temperatures were found to differ for the different samples and to range from 53.9 to 61.5°C.(129 to 142.7°F.) for the first loss of anisotropy and from 63.4 to 72.3°C.(146 to 162.2°F.) for the final disappearance of anisotropy. Among replicate samples, however, the values fell within 3 or 4°C. of each other. There appeared to be a negative correlation between the mealiness of the potatoes and both the temperature at first loss of anisotropy and the temperature at complete loss of anisotropy.

TABLE 2

Rigidity and Penetration Values of Gels Prepared at Various Final Temperatures

Sample No.	Final temperature (°C.)							Break in heating curve
	98	95	90	85	80	75	70	
	Rigidity (dynes 100 per sq. cm.)							
1	102	118	135	146	154	173	180	199
2	112	143	165	191	192	186	217	238
8	146	....	....	227	....	....	315	388
10	67	....	85	....	104	....	132	216
12	93	86	112	115	121	134	163	220
13	95	106	111	108	....	138	....	233
21	177	....	....	225	....	....	292	362
22	96	....	114	....	140	....	170	267
	Penetration (grams)							
1	110	123	149	158	150	170	173	166[1]
2	106	131	164	155	168	153	194	171[1]
8	136	....	....	148	....	....	212	186[1]
10	126	....	120	....	122	....	145	141[1]
12	105	124	127	124	130	133	139	166[1]
13	123	119	118	108	....	120	....	151[1]
21	128	....	....	146	....	....	174	196[1]
22	111	....	129	....	139	....	143	161[1]

[1] These values are probably low; the gels contained many small air bubbles.

Sixteen-per cent suspensions of starch in water were heated in a boiling-water bath. The temperature at which the suspensions suddenly thickened ranged from 64 to 68.5°C.(147.2 to 155.3°F.). The entire paste became translucent at temperatures ranging from 65.1 to 70.8°C.(149 to 159.5°F.). A break in the heating curve occurred coincident with the sudden thickening of the suspensions, indicating that gelatinization of the starch was accompanied by an endothermic reaction which took place at a temperature characteristic of the sample. The temperatures at which these three changes took place were different for each sample, regardless of varietal similarities. There was a tendency for the three changes to occur at lower temperatures with the starches from the more mealy potatoes.

The rigidity of cooled starch pastes varied from 61 x 10^2 to 286 x 10^2 dynes per square centimeter and penetration values ranged from 84 to 152

grams. Rigidity and penetration values for gels tended to increase as the final temperature to which the suspensions were heated decreased. The correlation coefficients between rigidity and mealiness indicate that more rigid gels were obtained from the starch of the less mealy potatoes. Penetration values were apparently unrelated to degree of mealiness.

The results of this study indicate that degree of mealiness of cooked potatoes may be associated with some of the physical properties of the starch of the raw tubers.

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PRODUCING DEHYDRATED MEATS BY VACUUM-ROTARY, PLATE-VACUUM, AND KETTLE-CABINET METHODS[1]

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Fundamentally the emergency meat-dehydration investigations of the United States Department of Agriculture, conducted in 1942 and 1943, were concerned with different processes. Among those studied were eight general methods identified as vacuum-rotary, plate-vacuum, kettle-cabinet, drum-cabinet, retort-cabinet, air-rotary, drum-air flotation, and freezing cabinet. This article presents certain results from studies of the first three of these methods, preceded by brief discussions of the meat used and yields of raw-lean and dehydrated product. According to plans the next article will be concerned with the other five dehydration processes.

MEAT USED

Beef, pork, lamb, and mutton were the meats used in the investigations. For the most part, the beef was obtained from heifers and dry cows culled from the beef and dual-purpose type Shorthorn herds at the Beltsville (Md.) Research Center of the Department. They ranged in age from two to 12 years and weighed from approximately 1,000 to 1,400 pounds on foot. The majority were Commercial in grade. The dressed carcasses were chilled and stored at 0.6 to 1.7°C. (33 to 35°F.) for 72 hours or more and were then quartered and boned. Promptly after boning, the meat was returned to the storage room where it was kept until needed. In no case did more than 14 days elapse between slaughter and dehydration. It was customary to bone an entire side at one time and thoroughly mix the meat in order to obtain uniform lots, representative of the entire dressed carcass, for processing. When dehydrated products of high-fat content were desired, little fat was trimmed from the lean, whereas all fat possible was removed from the raw meat if low-fat products were called for.

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[1] This is the second in a series of papers dealing with the emergency meat-dehydration investigations of the United States Department of Agriculture. The first article, entitled "Processing Equipment Used in Emergency Meat-Dehydration Research by United States Department of Agriculture," appears on pages 379-391 of this issue of *Food Research*.

[2] Resigned.

[3] Formerly with the Agricultural Research Administration, United States Department of Agriculture.

[4] The authors wish to give credit to R. L. Hiner of the staff of the Bureau of Animal Industry for supervising the slaughter of the animals and preparation of the meat for processing.

The pork used was largely from hogs produced and slaughtered at the Beltsville Research Center. The majority of the carcasses were graded as Choice Meat with a fair representation of Choice Fat and some of the lower grades. These hogs were from five to seven months old when slaughtered and had final feedlot weights of approximately 225 pounds. Some meat was used from Utility and Cull hogs weighing 75 to 150 pounds, discarded sows ranging from 200 to 600 pounds, Choice and Good commercial carcasses, and Choice commercial cuts. All carcasses were chilled at approximately 35°F. For drying, meat from hams, loins, picnic shoulders, shoulder butts, and lean trimmings was used, in the proportions occurring in the carcass. Thus the lots of meat as processed were always representative of the dressed carcass as a whole except that the head, fat back, belly (bacon), leaf fat, plate, fat trimmings, feet, and ribs were omitted. Generally, the extent to which the fatty tissue was trimmed from the lean was governed by the fatness desired in the finished produet. As a rule the meat was dehydrated the day after boning and trimming.

The lamb meat that was dehydrated came from animals ranging from approximately 55 to 100 pounds, live weight, and about 10 months old. Varying from Cull to Good, the lambs in large part were Commercial in grade. Three- to four-year-old ewes and wethers produced the mutton that was used in the work. Their live weights varied from about 110 to 180 pounds and their grades from Utility to Good, the majority in this instance also being Commercial. All lamb and mutton carcasses were chilled at about 35°F. and stored at that temperature until boned or cut into pieces in preparation for the actual processing. Meat from all parts of the dressed carcasses was included in the material for dehydration.

YIELDS OF RAW-LEAN AND DEHYDRATED MEATS

The yield of lean meat from the cattle used in this work was approximately 33 to 35 per cent of their live weight and 57 to 59 per cent of their dressed-carcass weight. The corresponding yields of pork lean from the cuts mentioned above were 26 to 28 per cent and 34 to 36 per cent; those of lamb were 26 to 28 per cent and 57 to 59 per cent.

Beef muscle of intermediate fatness, with the external and intermuscular fats carefully trimmed away, contained about 75 per cent water and 25 per cent dry matter. Provided that meat of this composition was dried without fat or other losses to a moisture content of 10 per cent, the yield of dehydrated meat from 100 pounds of raw meat would be about 27.8 pounds. With a moisture content of five per cent, the yield would be 26.3 pounds. However, the higher the proportion of fat in the raw material that is to be dried, the higher the yield of dehydrated meat will be: for example, it is estimated that beef containing 15 per cent trimmable fat will yield 34 pounds of dry matter per 100 pounds of raw material. On the 10-per cent moisture basis the corresponding yield of dehydrated beef would be 37.8 pounds.

VACUUM-ROTARY PROCESS

Work involving the vacuum-rotary process was done with three machines, Types A, B, and C, described in the first paper. The common characteristic of this process was drying under vacuum, with rotary agi-

tation, provided either by blades or paddles, or by revolution of the drying chamber itself.

Vacuum-Rotary Dehydrator A: Machine A was studied the most thoroughly of the three examined. Variables involved in the dehydration of beef, pork, and mutton were fatness of the raw meat, particle size, methods of pretreatment, effect of load, and effect of agitation.

General procedure for dehydrating by vacuum-rotary drier A consisted in grinding the boned, trimmed meat through a plate having holes of a certain diameter and placing 150 to 300 pounds of the material into the machine. The shaft to which the agitator blades were attached was set to turn at three revolutions per minute. A pressure of 15 to 30 pounds of steam was used in the steam jacket to heat the meat, while the heater-blower device removed, through the open-top vent, the moisture vapor that came from the meat. After about 70 minutes, when the free moisture had been removed, the machine was closed and the meat was dried under 29 inches of vacuum at 48.9 to 68.3°C.(120 to 155°F.). As the meat became drier the steam pressure in the jacket was reduced to prevent scorching. After four to five hours the meat was ready for removal from the drying chamber.

Results from 22 samples (Table 1) show that the fat content of the raw meat had no appreciable effect on drying rate. High-fat meat dried as rapidly as low-fat meat. In fact, the first and third series of pork samples indicate that it dried more rapidly. An explanation may be found in the larger amount of fat acting as a heat-transfer agent from the steam-heated shell to the meat particles, and the fact that there was a smaller proportion of water to be removed.

Another interesting fact brought to light (Table 1) is that larger sized pieces of meat of low-fat content took longer to dry than did smaller pieces but there was no difference between small and large pieces when the fat content of the raw meat was 10 to 12 per cent. Again, it appears that the fat acted as a heat conductor.

A number of comparisons of 100 and 73.9°C. (212 and 165°F.) as precooking temperatures which can be made between samples shown (Table 1), including those shown in pairs at the bottom, bring out the fact that on the whole the higher temperature resulted in about 1.25 hours less vacuum-drying time being required. This is explained by the fact that a larger proportion of the moisture was removed during the precooking period when the temperature was 212°F.

The rate of drying of meat under a 29-inch vacuum was greatly influenced by the temperature of the jacket of the dehydrator; for example, beef precooked at 212°F. subsequently dried to 10-per cent moisture content in about 2.5 hours with a jacket temperature of 134.4°C.(274°F.) (30 pounds' steam pressure), whereas a similar sample required about five hours when the jacket temperature was 106.1°C.(223°F.) (three pounds' steam pressure).

In a comparison of methods of precooking, two lots of meat were cooked for 70 minutes under an 18-inch vacuum at 165°F., whereas two other lots were cooked for the same period under atmospheric pressure at 160°F., utilizing the heater-blower attachment to remove moisture vapor. The two

TABLE 1

Drying Time for Meat Dehydrated by Vacuum-Rotary Process as Affected by Fat Content of Raw Meat, Particle Size, and Temperature of Precooking

Kind of meat	Sample No.	Fat content of raw meat	Particle size (diameter of holes in grinder plate)	Precooking		Temperature during drying	Time required to reduce moisture content to 10 per cent
				Temperature of meat	Length of period		
Effect of Fat Content							
		pct.	*in.*	*°F.*	*min.*	*°F.*	*min.*
Beef, Series 1	488	9	1.125	165	70	.....	278
	528	4	1.125	165	70	.....	281
Beef, Series 1	489	9	1.125	212	70	.....	224
	527	4	1.125	212	70	.....	230
Mean drying time, fat content of raw meat 9 per cent							251
Mean drying time, fat content of raw meat 4 per cent							256
Pork, Series 2	430	7-9	0.250	212	75	120-150	240
	444	7-9	0.250	212	75	128-150	240
Mean							240
Pork, Series 2	460	10-13	0.250	212	85	115-160	245
	504	10-13	0.250	212	70	125-150	290
	564	10-13	0.250	212	70	140-160	116
	532	10-13	0.250	212	70	120-160	200
Mean							213
Pork, Series 2	473	18	0.250	212	70	135-160	150
Pork, Series 2	788	27	0.250	200	60	120-160	120
Pork, Series 3	439	7-9	0.250	165	75	115-150	315
	445	7-9	0.250	165	70	108-160	265[1]
	553	7-9	0.250	165	75	100-158	284
Mean							288
Pork, Series 3	466	10-14	0.250	165	85	100-148	302
	503	10-14	0.250	165	70	100-150	290
	505	10-14	0.250	165	70	90-153	350
	565	10-14	0.250	165	70	105-170	255
Mean							299
Pork, Series 4	552	9	0.250	165[2]	70	95-160	299
Pork, Series 4	502	14	0.250	165[2]	70	98-150	287
Pork, Series 4	622	22	0.250	165[2]	70	128-165	230
Effect of Particle Size							
Beef, Series 5	449	4	0.250	212	70	120-150	155
	527	4	1.125	212	70	110-148	230
Beef, Series 5	452	4	0.250	165	70	108-150	270[1]
	528	4	1.125	165	70	105-152	281
Mean difference in drying time							43
Pork, Series 6	532	10	0.250	212	70	120-160	200
Pork, Series 6	548	11	0.875	212	70	120-155	200
Pork, Series 6	486	12	1.125	212	70	140-150	200

[1] Estimated. [2] Under vacuum.

TABLE 1 *(Concluded)*

Kind of meat	Sample No.	Fat content of raw meat	Particle size (diameter of holes in grinder plate)	Precooking		Temperature during drying	Time required to reduce moisture content to 10 per cent
				Temperature of meat	Length of period		
EFFECT OF TEMPERATURE OF PRECOOKING							
		pct.	*in.*	*°F.*	*min.*	*°F.*	*min.*
Beef, Series 7	490	8	0.250	212	70	130-155	275[1]
	492	6	0.250	165	70	100-150	299
Beef, Series 7	509	9	0.250	212	70	128-160	221
	510	9	0.250	165	70	105-155	281
Mean difference in drying time							41
Pork, Series 8	493	15	1.125	212	70	125-160	209
	494	15	1.125	165	70	110-155	254
Difference in drying time							45

vacuum-precooked samples dried in 293 minutes, while the air-precooked samples dried in 287 minutes. With this small difference in time it would seem advisable to carry out the precooking and drying under vacuum, since this method would minimize fat-oxidation damage.

A further study involved comparison of pork precooked at 104.4°C. (220°F.) and similar meat precooked at 73.9°C.(165°F.), with the use of the heater-blower attachment to remove moisture. The former lot required seven hours of drying time under vacuum as compared with five hours for the latter. The greater length of time in the case of the pressure-cooked meat was due to there being no moisture reduction during the precooking. All moisture had to be removed by the vacuum pump.

The economy of any drying operation is greatly influenced by the output of dehydrated product per unit of time. A summarization of data obtained in the investigations shows that 50-pound and 100-pound lots of meat could be processed in about the same length of time in vacuum-rotary dehydrator A. With little difference between them, likewise, 150-pound and 300-pound lots required less than twice as long. In 100-pound lots, one pound of meat required from 1.2 to 1.4 minutes of vacuum drying to attain a moisture content of 10 per cent, whereas in 300-pound lots only 0.8 minute was required.

A sample of mutton dehydrated by "standard" procedure, consisting of 212°F., 70-minute cooking followed by drying in 29-inch vacuum, indicated that this kind of meat would dehydrate as quickly as would pork of the same fat content under identical conditions.

Vacuum-Rotary Dehydrator B: Dehydrator B of the vacuum-rotary type was constructed basically like Drier A, but its capacity was about 40 pounds. The raw ground meat was heated by the steam-jacketed shell and the internally heated agitator which rotated at the rate of 5.6 r.p.m. The agitator blade had a clearance of less than 0.01 inch, so that during processing the disintegration of the meat particles was kept at a minimum. Precooking was carried out with the top of the machine removed. After

a suitable cooking period the top was replaced and the meat was dried under vacuum up to 29 inches.

Several preliminary runs indicated that a low steam pressure must be used to prevent scorching of the product. When four to 10 pounds of pressure was used in the jacket and agitator blade, the meat dried to 10 per cent moisture under 29 inches of vacuum in about two and one-half hours. However, the dried meat had a scorched appearance and odor. Suitable steam pressure was determined to be approximately one pound. The accompanying, relatively low jacket temperature resulted in the production of satisfactory dried meat in three and one-half hours.

Vacuum-Rotary Dehydrator C: Vacuum-rotary Dehydrator C differed essentially from the other vacuum-rotary driers in its method of agitation of the meat during the drying period. Instead of being equipped with internal agitators the entire steam-jacketed, vacuum-drying chamber rotated slowly. It was hexagonal in shape, and during operation the meat constantly tumbled from one flat surface to the next. Its capacity was about 20 pounds.

From a series of six experiments with pork and one with beef, the following conclusions were drawn. The most promising operating conditions were to fill the machine about one-half full of coarsely ground meat and to cook the meat under 18 inches of vacuum (about 165°F.) for 30 minutes. Dehydration was then carried out at 29-inch vacuum, with four pounds of pressure in the steam jacket until the meat temperature rose to 57.2°C.(135°F.). As the drying progressed into the latter stages of the operation, the temperature of the steam jacket was decreased in order to prevent the meat temperature from rising above 65.6°C.(150°F.) and the meat from scorching. Meat ground through the 1.125-inch plate was used successfully but that ground through the 0.25-inch plate formed into large balls and required more than five hours to dry to 10-per cent moisture content.

PLATE-VACUUM PROCESS

The plate-vacuum dehydrator, described in detail in the first article of this series, had two distinct advantages. The meat could be dried in the absence of any appreciable amount of air at low or high temperatures; and once placed in the machine, the meat particles remained concise since no agitation was used.

The experiments were carried out in three series with raw, cooked, ground, and unground meat. In Series 1, the meat was frozen and dried under vacuum; in the second series the meat was alternately frozen and heated during vacuum drying; and in Series 3 the meat was heated during vacuum drying. The second process involves what is known in industry as "flashing."

Series 1: Series 1 was carried out to show the effect of low-temperature, vacuum drying on rate of dehydration. One sample of pork was divided into two subsamples, each 0.75-inch thick and with relatively large surface area, one of which was raw and the other cooked. Within a few hours after the meat was placed on the plate of the drier its temperature was approximately —12.2°C.(10°F.) whereas that of the surrounding vacuum

chamber was 6.1 to 10°C.(43 to 50°F.). In a 29-inch vacuum for approximately 42 hours, under the temperature conditions mentioned, the raw meat lost little moisture and the cooked sample still contained 41 per cent moisture.

Another sample of pork was subdivided into parts as follows: (1) raw, unground piece, 0.75-inch thick; (2) similar to (1) but cooked; (3) raw, ground meat; and (4) cooked, ground meat. After remaining in the dehydrator for 40.5 hours under the same conditions as outlined in the preceding paragraph, the moisture percentages of the respective subsamples were 43, 36, 44, and 29.

A third sample of pork was ground through the 0.25-inch plate and divided into two parts, one of which was cooked and the other left raw. These two subsamples were processed under the same conditions as outlined above, except that the meat temperature was approximately —9.4°C. (15°F.). After 16.5 hours in the dehydrator the raw meat contained 54 per cent moisture and the cooked meat 48 per cent.

Series 2: Again, a sample of pork 0.75-inch thick was subdivided; one portion was cooked and the other portion was used uncooked. Both portions were placed in the plate-vacuum dehydrator, brine was circulated through the plates to freeze the meat to a temperature of approximately 10°F., and a 29-inch vacuum was applied. During this first period the temperature of the vacuum chamber ranged from approximately 43 to 50°F. After seven hours the brine was removed from the plates and steam introduced for one hour. Then the meat was again frozen by running brine through the plates. This same cycle was repeated 7.5 and 15 hours later. During the hour that the steam circulated through the plates, the temperature of the chamber was 37.8°C.(100°F.). After 25 hours of such operation the raw meat had a moisture content of 62 per cent and the cooked meat, 15 per cent. The meat was dark and scorched in appearance.

Ground pork, cooked and raw, was frozen and dried under a 29-inch vacuum for 5.5 hours, then heated for one hour as outlined above, and this cycle repeated 7.5 and 16 hours later. During the three heating or "flashing" periods, the vacuum chamber and internal meat temperatures were, respectively, 50 and 30°C.(122 and 86°F.), 60 and 25°C.(140 and 77°F.), and 70 and 35°C.(158 and 95°F.). The raw meat contained 30 per cent and the cooked meat 12 per cent moisture after 23.5 hours' drying. Since this meat also had a rather dark appearance, in the next sample the internal temperature of the meat was kept down to a 22.2°C.(72°F.) maximum. After 33 hours of drying with an hour "flash" period every 6.5 to 7.5 hours, the raw meat had 41 per cent moisture and the cooked meat had 18.5 per cent.

Series 3: The first lot of dehydrated pork in Series 3 was produced by using a vacuum-chamber temperature ranging from 90 to 107.2°C.(194 to 225°F.) and a vacuum that averaged 13.6 inches. The internal temperature of the meat (ground) varied from 48.9 to 86.7°C.(120 to 188°F.). A moisture content of 5.1 per cent was attained in four hours. The product was dark brown in color.

The next lot was dehydrated in a 20-inch vacuum, with the internal temperature of the meat not exceeding 71.1°C.(160°F.). Time required

a suitable cooking period the top was replaced and the meat was dried under vacuum up to 29 inches.

Several preliminary runs indicated that a low steam pressure must be used to prevent scorching of the product. When four to 10 pounds of pressure was used in the jacket and agitator blade, the meat dried to 10 per cent moisture under 29 inches of vacuum in about two and one-half hours. However, the dried meat had a scorched appearance and odor. Suitable steam pressure was determined to be approximately one pound. The accompanying, relatively low jacket temperature resulted in the production of satisfactory dried meat in three and one-half hours.

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Series 2: Again, a sample of pork 0.75-inch thick was subdivided; one portion was cooked and the other portion was used uncooked. Both portions were placed in the plate-vacuum dehydrator, brine was circulated through the plates to freeze the meat to a temperature of approximately 10°F., and a 29-inch vacuum was applied. During this first period the temperature of the vacuum chamber ranged from approximately 43 to 50°F. After seven hours the brine was removed from the plates and steam introduced for one hour. Then the meat was again frozen by running brine through the plates. This same cycle was repeated 7.5 and 15 hours later. During the hour that the steam circulated through the plates, the temperature of the chamber was 37.8°C.(100°F.). After 25 hours of such operation the raw meat had a moisture content of 62 per cent and the cooked meat, 15 per cent. The meat was dark and scorched in appearance.

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The next lot was dehydrated in a 20-inch vacuum, with the internal temperature of the meat not exceeding 71.1°C.(160°F.). Time required

to dry to 10.3 per cent moisture was six hours. When the vacuum was increased to 29 inches, other conditions remaining the same, 7.1-per cent moisture content was attained in 4.5 hours. With a 29-inch vacuum and the internal temperature of the ground pork not exceeding 60°C.(140°F.), six hours were required to dehydrate to a moisture content of 5.1 per cent. All samples in this series heated to more than 140°F. internal temperature showed some scorching.

The results from the three series of lots, which involved the use of the plate-vacuum dehydrator in their processing, indicate that when meat is frozen or alternately frozen and heated and the vacuum is no more than 29 inches, the operation is too time-consuming to be practical. Heating the product during dehydration greatly accelerated the drying but it appeared that to avoid scorching, an internal meat temperature of 140°F. should not be exceeded.

KETTLE-CABINET PROCESS

In any consideration of possible methods for the dehydration of meat, thought will turn, sooner or later, to the kettle-cabinet process. The steam-jacketed kettle is used for precooking and the cabinet for drying proper. From the viewpoint of practical application the cabinet drier may be regarded as such, or in terms of a tunnel drier equipped with tray trucks or a conveyor. In the experiment described below, use was made of a steam-jacketed kettle and cabinet dehydrators A and C described in the first paper of this series on meat dehydration.

Studies were conducted on the following factors: (a) fat content of raw meat, (b) particle size of meat, (c) temperature of precooking, (d) time of grinding, before and after precooking, and (e) temperature of drying.

General operations consisted in cooking the meat at a predetermined temperature (165 to 212°F.) until all free moisture had evaporated, grinding into 0.25- to 1.125-inch particles, spreading onto trays at the rate of 1.5 pounds per square foot of tray surface, and drying in moving air at the desired temperature.

Cabinet Dehydrator A: In a series of experiments concerning the effect of fat content on the rate of drying, two beef samples and two pairs of pork samples were dried at 160°F. As shown (Table 2) beef samples contained eight and 12 per cent fat on the raw basis, and the pork samples contained nine and 13 per cent fat in the case of each pair. The drying time for the low-fat beef sample was 205 minutes as contrasted with 260 minutes for the fatter sample. The low-fat pork samples dried in an average of 216 minutes, whereas the fatter pork samples required 377 minutes. The moisture content of the finished product in all cases was six per cent.

Size of meat particle, as determined by the diameter of the holes in the grinder plate, was found to be an important factor governing the rate of dehydration. Eight samples of lamb and mutton are also shown (Table 2). In pairs, one sample was ground through the 0.25-inch plate and the other through the 0.875-inch plate. In all instances the meat of the smaller size particles dried to 10-per cent moisture content much more rapidly than that of larger size particles. This was true despite the fact, in the

cases of the two pairs of mutton samples, that the meat in larger size particles was much the less fat. It will be noted that the air temperatures (dry bulb) were 65.6 and 82.2°C.(150 and 180°F.).

That precooking at 212°F. in comparison with 165°F. resulted in little difference in subsequent rate of drying is further shown (Table 2).

In preparing meat for dehydration it may be ground either before or after cooking. Results obtained in the investigation showed a tremendous saving in time required for dehydration when the meat was ground after cooking just prior to spreading on the drying trays. In the cases of three pairs of beef samples, the mean drying time of those ground before cooking was 140 minutes as compared with 77 minutes for corresponding samples ground after having been cooked. Equally striking differences, in favor of grinding after cooking, were shown by a larger number of pork samples.

In a study of drying temperature, lamb meat of 10-per cent fat content ground through a 0.25-inch plate, precooked at 212°F. for 95 minutes, and dehydrated at 180°F. (dry bulb), with 31 per cent relative humidity in the drying chamber, was reduced to 10-per cent moisture content in 150 minutes following the cooking. Other lamb meat, similarly ground and cooked but dehydrated at 150° F. in an atmosphere of the same relative humidity required 180 minutes to reach a moisture level of 10 per cent, although the fat content of the raw material was only five per cent. With a similar pair of lamb samples, processed under the same condition except that a 0.875-inch grinder plate was used, the meat dehydrated at the higher temperature required five hours, whereas that dehydrated at the lower temperature required eight hours.

Two pairs of mutton samples were processed in a similar manner. One pair, containing approximately 20 per cent fat and ground through the 0.25-inch plate, dried to the 10-per cent moisture level in 140 and 180 minutes at 180 and 150°F., respectively. The samples in the other pair, containing six per cent fat and ground through the 0.875-inch plate, were reduced to 10-per cent moisture content in 240 and 300 minutes at the drying temperatures of 180 and 150°F., respectively.

Considering the four pairs of lamb and mutton samples, drying at 180°F. rather than 150°F. saved from 30 to 180 minutes.

To throw light on the effect of utilizing more than one temperature in cabinet drying, a relatively large lot of pork of 3.25-per cent fat content was subdivided into three samples, each ground through the 0.25-inch plate and precooked for 30 minutes at 212°F., and the dehydration carried out as follows:

Sample 1. 180°F. for 120 minutes, 150°F. for 120 minutes
Sample 2. 200°F. for 60 minutes, 150°F. for 60 minutes
Sample 3. 200°F. for 30 minutes, 180°F. for 30 minutes, 150°F. for 240 minutes.

The respective moisture percentages in the finished products were 11.8, 10.5, and 8.5. Most economical in time, of the three methods studied, was that utilizing 200°F. air for one hour followed by 150°F. air for another hour. Moreover, in view of the more rapid evaporation rate during the early part of the drying period when the meat had a higher moisture content, resulting in a low wet-bulb or meat temperature, there was less

TABLE 2

Drying Time for Meat Dehydrated by Kettle-Cabinet Process as Affected by Fat Content of Raw Meat, Size of Particle, and Temperature of Precooking

Kind of meat	Sample No.	Fat content of raw meat	Particle size (diameter of holes in grinder plate)	Precooking		Temperature of drying (dry bulb)	Time required to reduce moisture content to 10 per cent	Total drying time	Moisture content of dehydrated product
				Temperature of meat	Length of period				
Effect of Fat Content									
		pct.	*in.*	*°F.*	*min.*	*°F.*	*min.*	*min.*	*pct.*
Beef	366A	8	.375	212	30	160	.....	205[1]	6.0
	365	12	.375	212	30	160	.....	260[1]	6.0
Pork	398	9	.250	165	30	160	.....	252[1]	6.0
	411	13	.250	165	30	160	.....	370[1]	6.0
Pork	399	9	.250	212	30	160	.....	180[1]	6.0
	412	13	.250	212	30	160	.....	385[1]	6.0
Effect of Size of Particle									
Lamb	607	10	.250	212	95	180	150	.....	....
	608	9	.875	212	95	180	300	.....	....
Lamb	609	5	.250	212	95	150	180	.....	....
	610	5	.875	212	95	150	480[1]	.....	....
Mean difference	.....	....	.....	.....	....	....	225	.....	....
Mutton	578	22	.250	212	95	180	140	.....	....
	580	6	.875	212	95	180	240	.....	....
Mutton	579	19	.250	212	95	150	180	.....	....
	581	6	.875	212	95	150	300	.....	....
Mean difference	.....	....	.....	.....	....	.....	110	.....	....
Effect of Temperature of Precooking									
Beef	364	14	.375	165	30	160	.....	257[1]	9.0
	365	12	.375	212	30	160	.....	225[1]	9.0
Pork	442	7	.250	165	40	180	.....	120	6.7
	441	7	.250	212	40	180	.....	120	7.1
Pork	411	13	.250	165	30	160	.....	360	6.7
	412	13	.250	212	30	160	.....	360	7.4
Pork	401	14	.250	165	30	160	.....	180	6.8
	402	14	.250	212	30	160	.....	180	[1]6.6

[1] Estimated.

danger of damage to the product from using 200°F. air at that time than if it had been used later during the drying operation.

Question often arises as to consistency of results from a given drying operation. One lot of beef containing six per cent fat was divided into four uniform lots. Each sample was ground through the 0.25-inch plate, precooked at 165°F. for 30 minutes, spread on trays at the rate of 1.5 pounds of precooked meat per square foot, and dried at 160°F. for four hours. The moisture contents of the dehydrated products were 6.7, 7.3, 5.7, and 6.6 per cent. These values suggest that there should be no great trouble in producing consistent results under well-controlled, practical operating conditions.

Cabinet Dehydrator C: In a study of cabinet Dehydrator C, pork in two-inch cubes was first precooked at 212°F. for 40 minutes. After the cooking was partially completed, the free liquors and melted fat were drained from the meat, the fat separated from the juice, the juice added back to the solid material, and the cooking continued for the total period mentioned. The precooked meat was then ground through the 0.25-inch plate, spread in shallow enamelled pans at the rate of 1.5 pounds per square foot, and dried at 180°F. In 135 minutes the product was dried to seven per cent moisture content. The fat content of the dehydrated meat was 30.9 per cent.

DISCUSSION

There was a number of readily apparent advantages in using the vacuum-rotary process for meat dehydration. In the first place, both the precooking and drying operations were carried out in the same piece of equipment, resulting in certain obvious economies. Meat of moderate and high fat content was dried as rapidly as meat of lower fat content. Large meat particles dried as quickly as did smaller size particles. The largest load of meat in the machine which caused no undue mechanical strain, resulted in maximum production per unit of time. The meat was protected from air contact during that stage of the operation in which greatest injury owing to fat oxidation might occur.

Dehydrated meat could be produced by the vacuum-rotary method in 3.5 to 4.5 hours of drying time.

When meat was frozen or alternately frozen and heated in plate-vacuum dehydration, with the vacuum no more than 29 inches, the processing was too slow to be practical. Continuous heating greatly accelerated the drying but an internal meat temperature higher than 140°F. appeared to scorch the product. When dehydrated by this method, meat particles remained concise and whole.

In the kettle-cabinet process the rate of dehydration was materially reduced when the fat content of the meat was high and when the particle size was large.

The use of air heated to 180°F. resulted in a satisfactory rate of drying. It appeared feasible to use 200°F. air for short periods of time, when the meat was still in the wet stage.

When dehydration is done by the kettle-cabinet method, it would seem advisable to use meat containing about 15 per cent fat on the raw basis. Meat cooked in small pieces about two inches in size, ground through the

0.25-inch plate, placed onto trays at the rate of 1.5 pounds per square foot, and dried at 180°F. was reduced to a moisture content of 10 per cent in about two to four hours.

CONCLUSION

Meat can be satisfactorily dried by the vacuum-rotary or the kettle-cabinet method. Each method has certain advantages.

In using the vacuum-rotary method the rate of drying is not dependent on fat content, particle size, or atmospheric conditions.

The kettle-cabinet method produces a more concise piece of dried meat, and the process can, with some adaptation, be made continuous.

CONSTITUENTS OF CRUDE CAROTENE OF CARROTS

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Carrots have been known for some time to be a rich source of carotene, Wackenroder (1931); Bills and McDonald (1932); and Barnes (1936). Kuhn and Lederer (1941) found that besides beta-carotene, the carotene of carrots consisted of 10 to 20 per cent alpha-carotene. Strain (1939) showed also that carrots contained alpha carotene. Recent work by Kemmerer and Fraps (1943, 1944) showed that the carotene extracts of plants are complex in nature and may consist of beta-carotene, of impurity A, of neo-beta-carotenes B and U, and sometimes of alpha-carotene. The neo-beta-carotenes are stereoisomers of beta-carotene and can be formed from and converted to beta-carotene. Neo-beta-carotene B and alpha-carotene have one-half the vitamin A activity of beta-carotene while impurity A does not appear to possess vitamin A activity. Kemmerer and Fraps (1943) report that neo-beta-carotene U does not possess vitamin A activity, while Deuel, Johnston, Sumner, Polgar, and Zechmeister (1944) report that it has 38 per cent of the vitamin A activity of beta-carotene.

Recently it was observed in this laboratory that the proportion of alpha-carotene was lower in some samples of dehydrated carrots received from the National Research Council than in dehydrated carrots prepared in Texas. Because of this observation and because of the recent findings on the complexity of natural carotene, it seemed desirable to determine the constituents of the crude carotene of a number of different samples of carrots.

EXPERIMENTAL PROCEDURE

Fresh carrots were collected from local markets. Dehydrated carrots grown in California, that were received from the National Research Council and from the University of California, and dehydrated carrots, grown and manufactured in Texas, were used. All the carrots were analyzed for the crude carotene constituents by methods already published by Kemmerer and Fraps (1943).

DISCUSSION OF RESULTS

The crude carotene content and the percentages of constituents of the crude carotene for the carrots are given (Table 1); this table also includes the percentages of beta-carotene equivalents—Kemmerer, Fraps, and Meinke (1945)—which are the percentage of beta-carotene plus one-half the alpha-carotene plus one-half the neo-beta-carotene B. This beta-carotene equivalent is a measure of the vitamin A-active carotenes. Carrots with a high percentage of alpha-carotene will have a lower beta-carotene equivalent than carrots having a low percentage of alpha-carotene. The percentages of constituents were calculated on the total amount of the

TABLE 1

Constituents of Crude Carotene of Carrots

Source and variety	Crude carotene	Impurity A	Percentage of constituents				Beta-carotene equivalent
			Neo-beta-carotene U	Beta-carotene	Neo-beta-carotene B	Alpha-carotene	
Fresh carrots (dry basis) from College Station	*p.p.m.*						
Imperator—early stage of growth	386	5.8	0.0	60.1	0.0	34.1	77.2
Imperator—late stage of growth	528	7.7	0.0	55.1	0.0	37.2	73.7
Purchased on market	36[1]	6.5	0.0	64.7	0.0	28.8	79.1
Purchased on market	698	2.7	3.5	73.3	0.0	20.5	83.6
Purchased on market	101[1]	6.2	0.0	53.1	2.1	38.6	73.5
Purchased on market	1036	6.2	0.0	52.1	5.6	36.1	73.0
Purchased on market	1120	3.3	1.3	58.8	4.0	32.6	77.1
Mean	754	5.5	0.7	59.6	1.7	32.5	76.7
Dehydrated carrots from National Research Council, grown in California							
Not known	750	7.1	2.5	62.7	4.0	23.7	76.6
Imperator	942	7.7	0.0	62.9	7.9	21.5	77.6
Chantenay	855	3.7	0.0	70.5	6.3	19.5	83.4
Not known	716	4.6	3.2	67.3	5.2	19.7	79.8
Mean	816	5.8	1.4	65.8	5.9	21.1	79.4
Carrots grown and dehydrated in Texas							
Danvers Half Long	238	14.7	0.0	44.9	5.2	35.2	65.1
Amsterdam Forcing	247	13.7	0.0	49.6	0.0	36.7	68.0
Amsterdam Forcing	312	14.3	0.0	52.7	1.3	31.7	69.2
Chantenay No. 3	255	19.2	0.0	40.5	1.2	39.1	60.7
Red Core Chantenay	363	10.8	3.7	50.3	4.0	31.2	67.9
Imperator	348	11.4	0.0	51.3	0.0	37.3	70.0
Imperator	424	13.3	8.6	42.1	0.0	36.0	60.1
Morses Bunching	320	16.0	0.0	45.0	0.0	39.0	64.5
Morses Bunching	910	6.1	0.0	55.0	4.4	34.5	74.5
Monteg	344	14.0	3.0	46.4	3.4	33.2	64.7
Long Orange	733	9.8	4.3	49.9	3.6	32.4	67.9
Nantes	659	7.8	4.0	49.3	3.4	35.5	68.8
Nantes	730	4.4	0.0	60.6	0.0	35.0	78.1
Luxemberg Half Long	629	5.7	0.0	63.3	2.3	28.7	78.8
Touchon	920	7.7	0.0	59.5	0.0	32.8	75.9
Louisiana Danvers	930	5.2	0.0	60.2	3.2	31.4	77.5
Supreme Half Long	720	4.2	0.0	59.5	5.3	31.0	77.7
Mean	535	10.4	1.4	51.8	2.2	34.1	70.0
Dehydrated carrots from University of California, 1941							
Morses Bunching	208	10.0	0.0	47.8	0.0	42.2	68.9
Danvers Half Long	165	12.6	2.8	45.5	5.5	33.6	65.9
Red Core Chantenay	171	18.4	2.3	49.7	2.7	26.9	64.5
Imperator	185	15.4	2.2	47.3	2.3	32.8	64.9
Nantes	207	8.0	1.7	57.2	3.1	30.0	73.7
Mean	187	12.9	1.8	49.5	2.7	33.1	67.4

[1] Carotene determination made on wet basis.

several pigments recovered after adsorption on a calcium hydroxide column as was done by Kemmerer and Fraps (1943) in previous work. In only a few cases was the total below 90 per cent of the crude carotene.

On the dry basis, the crude carotene content of the fresh carrots ranged from 386 to 1,120 p.p.m. with a mean of 754 p.p.m.; the dehydrated carrots from the National Research Council ranged from 750 to 942 p.p.m. with a mean of 816 p.p.m.; the dehydrated carrots grown and dehydrated in Texas ranged from 238 to 930 p.p.m. with a mean of 535 p.p.m.; and the dehydrated carrots from the University of California ranged from 165 to 208 p.p.m. with a mean of 187 p.p.m. The low carotene content of the latter may be due to the long period of storage (1941 to 1944). Impurity A appears to be slightly higher in the Texas dehydrated carrots and those from the University of California than in those received from the National Research Council. Neo-beta-carotenes B and U were present in low amounts in the four groups of carrots. The greatest difference was in the percentages of alpha carotene. In the dehydrated carrots from the National Research Council it ranged from 19.5 to 23.7 per cent with a mean of 21.1 per cent; in the fresh carrots the range was 20.5 to 38.6 per cent with a mean of 32.5 per cent; in the Texas dehydrated carrots the range was 28.7 to 39.1 per cent with a mean of 34.1 per cent; and in the dehydrated carrots from the University of California the range was 26.9 to 42.2 per cent with a mean of 33.1 per cent. These differences are not due to variety since the Imperator and Chantenay carrots from the National Research Council contained considerably lower percentages of alpha-carotene than did the same varieties of carrots from Texas. Differences in soil and climate are the most likely factors.

SUMMARY

Fresh carrots grown in Texas contained 386 to 1,120 p.p.m. crude carotene on the dry basis with a mean of 754 p.p.m. Dehydrated carrots from the National Research Council contained 750 to 942 p.p.m. with a mean of 816; dehydrated carrots from the University of California contained 165 to 208 p.p.m. with a mean of 187; and dehydrated carrots grown and dehydrated in Texas contained 238 to 930 p.p.m. with a mean of 535 p.p.m. The percentage of alpha-carotene in the dehydrated carrots received from the National Research Council ranged from 19.5 to 23.7 with a mean of 21.1; the percentage in dehydrated carrots from the University of California ranged from 26.9 to 42.2 with a mean of 33.1; the percentage in fresh carrots grown in Texas ranged from 20.5 to 38.6 with mean of 32.5; and the percentage in carrots grown and dehydrated in Texas ranged from 28.7 to 39.1 with a mean of 34.1.

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MAGNESIUM CONTENT OF MEATS

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Data on the protein, fat, carbohydrate, calcium, phosphorus, ash, and water content of duplicate diets given to two men on an exclusive meat diet have been reported by Toscani, Rupp, and McClellan (1934). As magnesium plays an important role in the mineral metabolism, it was deemed desirable to supplement the above data with the magnesium values for the meats used in the experiment.

EXPERIMENTAL PROCEDURE

There were 71 samples of meat, including several each of lean beef, medium cut beef, beef liver, beef kidney, veal, and lamb.

During the first three months of the experiment the meat used was obtained from animals slaughtered the preceding day. After the third month ordinary refrigerated meats were used. The meals given the subjects were duplicated as closely as possible as those sent to the laboratory. The meats were sent to the laboratory daily in enameled pails and preserved on ice for the duration of the period which was usually seven to 10 days. The meat was then sorted, weighed, ground in a meat chopper, and dried on a steam bath in porcelain evaporating dishes. When dry, the specimens were weighed, ground, and preserved in Mason jars.

For analysis, an aliquot portion of the stored sample was finely ground in a porcelain mortar, quartered, and samples taken for duplicate determinations; the composition of the various meats is given (Table 1).

The magnesium was determined on the filtrate after the removal of calcium. The following procedure, based on a modification of Shohl and Pedley (1922), was used for the separation of the calcium. Four grams of the dried meat is placed in a 500-c.c., long-neck Kjeldahl flask. The neck of the flask is washed down with a small portion of distilled water. Ten c.c. of concentrated sulphuric acid and five c.c. of concentrated nitric acid is introduced into the flask. The flask is gently heated until carbonization occurs. Then additional small portions of nitric acid are added until the contents of the flask assume a clear green color. The digested material is allowed to cool, diluted with water, neutralized with concentrated ammonium hydroxide and the calcium is precipitated according to Shohl and Pedley (1922).

The magnesium is determined on the filtrate from the calcium separation, according to McCrudden (1909). As the method involved both the Shohl and Pedley and McCrudden procedures, several recovery experiments of added magnesium to meat samples were run. The recovery of added magnesium is given (Table 2); the magnesium content of meats on a moist-weight basis is presented (Table 3).

TABLE 1

Composition of the Various Meats

Number of samples	Nature of meat	Protein	Fat	Carbohydrate	Water	Ash
		pct.	*pct.*	*pct.*	*pct.*	*pct.*
19	Beef muscle well trimmed					
	Max	21.80	7.60	1.49	74.90	1.38
	Min.	18.15	2.88	1.35	69.80	0.89
	Av.	20.19	5.60	1.36	71.80	1.06
10	Beef muscle, not trimmed					
	Max	22.50	20.60	1.38	69.90	1.23
	Min.	17.75	7.90	1.12	54.40	0.85
	Av.	19.85	13.13	1.32	64.70	1.00
19	Beef tongue					
	Max	26.00	34.60	1.53	68.00	1.18
	Min.	16.90	8.00	0.72	42.90	0.64
	Av.	19.85	23.60	1.09	54.50	0.89
13	Beef liver					
	Max	24.30	8.55	3.25	75.50	1.91
	Min.	15.30	4.20	1.70	65.80	1.35
	Av.	20.10	6.34	2.44	69.50	1.62
2	Beef kidney					
	Max	15.40	2.50	0.40	82.10	1.06
	Min.	13.90	2.40	0.40	80.70	1.10
	Av.	14.65	2.45	0.40	81.40	1.08
1	Beef brain	10.50	9.00	1.08	77.80	1.61
4	Lamb muscle					
	Max	16.00	33.60	0.76	59.70	0.99
	Min.	13.95	22.90	0.54	49.40	0.77
	Av.	15.14	27.00	0.69	56.40	0.85
3	Veal muscle					
	Max	18.80	17.20	1.62	65.50	1.10
	Min.	17.20	13.50	1.38	62.80	0.86
	Av.	17.80	15.96	1.50	63.80	0.96

DISCUSSION

While the individual samples of the various cuts of beef tissue gave large variations in the magnesium content on the moist-weight basis, the average figures (Table 3) agreed closely with the values found on beef muscle by Katz (1896), Bunge (1885), and Bertram (1878). The individual variations of magnesium on the moist-weight basis can probably be explained by the variation of the fat content of the tissues. Even in beef muscle, which was trimmed as carefully as possible to remove excess fat, the fat content ranged between 2.9 and 7.6 per cent. This was due to differences in the amount of fat lying between the strands and fibers of the muscle, which could not be removed by trimming. The amount of fat of this nature is dependent upon the food with which the animal was fattened.

Magnus-Levy (1910) reports magnesium on human material as follows: muscle 21.5 mg., liver 17.5 mg., kidney 20.7 mg., and brain 13.9 mg. The values for human muscle and liver are lower than those found in beef tissues. As only two samples of beef kidney and one sample of beef brain were analyzed, the data were insufficient for comparison with human tissue.

TABLE 2

Recovery of Added Magnesium

In beef	Added	Found	Recovered	Recovery
mg.	*mg.*	*mg.*	*mg.*	*pct.*
2.18	4.42	6.55	4.37	98.8
2.18	8.85	11.36	9.18	104.0
2.18	13.27	15.18	13.00	98.0
2.18	17.69	19.87	17.69	100.0

TABLE 3

Magnesium per 100 Grams of Meat, Moist Weight

Number of samples	Nature of meat	Magnesium			Mean deviation
		Maximum	Minimum	Average	
		mg.	*mg.*	*mg.*	
19	Beef muscle, well trimmed	31.6	19.8	24.9	0.63
10	Beef muscle, not trimmed	31.9	18.7	24.0	1.13
19	Beef tongue	30.0	12.6	21.9	1.00
13	Beef liver	28.6	14.6	22.0	1.05
2	Beef kidney	15.5	12.5	14.0	.....
1	Beef brain	.....	.....	13.0	.....
4	Lamb muscle	23.8	15.2	19.3	.....
3	Veal muscle	27.0	24.6	25.5	.....

TABLE 4

Magnesium in Ash

Number of samples	Nature of meat	Ash in 100 gm. fresh tissue			Magnesium in ash			Mean dev.
		Max.	Min.	Av.	Max.	Min.	Av.	
		gm.	*gm.*	*gm.*	*pct.*	*pct.*	*pct.*	
19	Beef muscle, well trimmed	1.38	0.89	1.06	2.74	1.86	2.34	0.20
10	Beef muscle, not trimmed	1.23	0.85	1.01	3.02	1.77	2.37	0.37
19	Beef tongue	1.18	0.64	0.89	3.85	1.45	2.51	0.59
13	Beef liver	1.91	1.35	1.62	1.78	0.91	1.31	0.23
2	Beef kidney	1.06	1.10	1.08	1.42	1.18	1.30	.....
1	Beef brain	.....	.....	1.61	.....	.....	0.81	.....
4	Lamb muscle	0.99	0.77	0.85	2.42	1.90	2.25	.....
3	Veal muscle	1.10	0.86	0.96	2.94	2.27	2.71	.....

Veal muscle showed the least variation in magnesium of the several samples analyzed. The magnesium content of the ash is given (Table 4). The mean deviations in Tables 3 and 4 were calculated according to Scott (1927) using the equation

$$\text{M.D.} = \sqrt{\frac{d^2}{n(n-1)}}.$$

SUMMARY

The magnesium content was determined on 71 samples of various cuts of meat. The average figures show close agreement with the published data.

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BLANCHING OF VEGETABLES FOR FREEZING. EFFECT OF DIFFERENT TYPES OF POTABLE WATER ON NUTRIENTS OF PEAS AND SNAP BEANS[1]

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Many studies have been conducted on the blanching of vegetables[2] in water prior to freezing to determine the losses of several of the important vitamins. Information on the effects of different types of potable water, such as hard water, water containing iron, and even city water rendered safe for use by the addition of chlorine, is lacking. The purpose of this study was to determine the effects of these different waters as compared with distilled water on the ascorbic acid, carotene, thiamin, riboflavin, niacin, calcium, and iron contents of peas and snap beans.

EXPERIMENTAL PROCEDURE

Vitamin C was determined by direct titration with sodium 2,6-dichlorobenzenoneindophenol, according to a modified Mack and Tressler (1937) method, employing eight per cent acetic acid in the titration; carotene by the extraction method of Zimmerman, Tressler, and Maynard (1940); and thiamin by the method of Moyer and Tressler (1942). Microbiological methods of Snell and Strong (1939) and Krehl, Strong, and Elvehjem (1943) were used for riboflavin and niacin. For the calcium determination, samples of the vegetables were wet-ashed with perchloric acid, precipitated with oxalate, and titrated with permanganate in the usual manner. The iron was estimated with o-phenanthroline, according to the method of Cowling and Benne (1942), on the wet-ashed samples.

The peas used in this study were of the Thomas Laxton variety which appears to yield the highest quality frozen product of any grown in this area. They were harvested and vined by machinery. They showed a tenderometer reading of 94, a value within the limits used by commercial producers of fancy peas. They were ungraded as to size, which is standard commercial and home-freezing practice.

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[1] This report is based on one of a series of studies supported largely by the Consolidated Edison Company of New York, Incorporated. The studies are being carried out under the direction of the Research Committee on Food Processing and Storage appointed from the staff of Cornell University, consisting of the following members: Dr. L. A. Maynard, chairman; Dean Sarah G. Blanding; Dr. C. E. F. Guterman; Dr. A. J. Heinicke; Dr. F. H. Rhodes.

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[2] Some of the vegetables used in this study were furnished by the Division of Vegetable Crops of this Station.

The beans were of the Tendergreen variety. This variety can be dependably grown in this area, both in home gardens and commercially, and yields a frozen product of good quality. They were snipped and cut into three-fourth inch segments by machinery.

For blanching, one gallon of water was used for each pound of vegetables treated. Since the work was done with comparatively small quantities on the scale of the home processor, the long flumes for washing and transportation and the brine separator used by the commercial producer were not employed. In place of running water for cooling, a volume of water equal to that used for the blanching was substituted. This was chilled and contained ice for cooling the vegetables after blanching.

The hard water was prepared in the laboratory from distilled water and chemically pure materials and contained 500 parts per million of calcium bicarbonate for temporary hardness and 100 parts per million of calcium sulfate for permanent hardness. Potable waters of this degree of hardness occur in this state. Water with chlorine was prepared from distilled water and contained 0.25 part per million of chlorine. The iron containing water was made with one part per million of iron.

The peas were blanched for 60 seconds in rapidly boiling water, the beans for two minutes. After the lots were blanched in each of the several types of water and cooled, samples were removed for immediate analysis. Samples of the blanch water were titrated for ascorbic acid immediately after the blanching of each lot was completed. The cooling water was also titrated in similar fashion. The blanched peas and beans were packed in moisture-vapor-proof containers and frozen in a freezing cabinet without a fan, to approximate home conditions. The temperature used was —17.8°C.(0°F.).

After six months' storage the samples were again analyzed. The stored samples were also cooked by a standardized procedure (Faith Fenton, personal communication) as follows: one-fourth cup of water was brought to boiling in a small covered saucepan five inches in diameter. This was large enough to accommodate the cake of frozen vegetable resting on the bottom. The flame was lowered after the water came to a boil the second time. The peas were cooked a total of eight minutes, the beans 10 minutes. The vitamin and mineral contents of the peas are recorded (Table 1). The related data on beans are recorded (Table 2).

DISCUSSION

It was found that 10 to 19 per cent of the ascorbic acid of peas was lost during the type of processing described. This is somewhat lower than the 30-per cent loss recorded by Jenkins, Tressler, and Fitzgerald (1938) during commercial processing. The greater loss found during commercial processing follows from conditions permitting greater leaching losses. On the other hand, Jenkins and co-workers recorded little loss during storage, while comparable storage in the present study resulted in moderate losses. Therefore, the ascorbic acid content of the stored peas, whether commercially processed or processed according to the method given in the present study, were essentially the same. It appears that a certain part of the ascorbic acid is especially susceptible to destruction, and if it is not lost

TABLE 1

Changes in Nutrients in Peas After Blanching in Different Types of Potable Water, After Storage for Six Months at —17.8°C.(0°F.), and After Cooking Following Storage[1]

Nutrient	Raw	Distilled water			Water with chlorine			Hard water			Water with iron		
		A[2]	B[2]	C[2]	A	B	C	A	B	C	A	B	C
Ascorbic acid, mg	.288	.260	.162	.142	.248	.161	.136	.234	.159	.157	.257	.160	.137
Carotene, gamma	4.22	3.80	3.61	4.06	3.78	3.30	3.58	4.00	3.57	4.31	4.10	4.18	4.22
Thiamin, gamma	3.45	3.17	2.86	2.74	3.20	2.89	2.74	3.03	2.81	3.29	2.97	2.83	3.20
Riboflavin, gamma	1.76	1.44	1.21	1.20	1.24	1.19	1.18	1.32	1.20	1.25	1.52	1.18	1.26
Niacin, gamma	23.3	21.6	20.7	20.3	22.2	21.9	21.0	22.8	22.6	22.9	23.2	22.9	24.3
Calcium, gamma	188	183	191	189	196	210	208	311	289	325	246	242	253
Iron, gamma	23.1	17.8	17.2	16.8	22.7	22.9	16.3	22.0	21.1	20.8	22.2	22.2	18.3
Solids, pct	22.2	21.6	21.1	22.6	21.1	20.6	21.6	21.2	21.8	24.5	21.4	21.1	25.3

[1]All results expressed on the per gram wet basis. [2]A—blanched; B—after six months' storage; C—after cooking following storage.

TABLE 2

Changes in Nutrients in Snap Beans After Blanching in Different Types of Potable Water, After Storage for Six Months at —17.8°C.(0°F.), and After Cooking Following Storage[1]

Nutrient	Raw	Distilled water			Water with chlorine			Hard water			Water with iron		
		A[2]	B[2]	C[2]	A	B	C	A	B	C	A	B	C
Ascorbic acid, mg	.232	.200	.125	.106	.197	.121	.112	.217	.121	.113	.214	.116	.110
Carotene, gamma	4.76	4.51	4.28	5.65	4.52	4.08	4.85	4.78	4.33	5.18	4.90	4.00	5.00
Thiamin, gamma	0.90	0.79	0.56	0.40	0.80	0.59	0.46	0.76	0.59	0.51	0.76	0.58	0.54
Riboflavin, gamma	1.20	1.00	1.03	1.00	0.94	1.00	0.99	1.03	0.96	0.98	1.09	0.94	0.92
Niacin, gamma	9.5	8.5	8.5	8.6	9.2	8.3	8.4	8.9	8.1	8.0	8.3	8.0	7.9
Calcium, gamma	436	417	396	457	434	407	461	451	491	514	396	470	418
Iron, gamma	11.0	10.3	8.5	9.8	9.9	9.9	10.2	8.3	8.5	10.1	9.6	9.5	10.3
Solids, pct	9.74	8.96	8.75	11.86	9.32	9.56	11.37	9.36	9.54	11.55	8.69	8.06	10.78

[1]All results expressed on the per gram wet basis. [2]A—blanched; B—after six months' storage; C—after cooking following storage.

during processing, it is lost during storage. A storage loss was also noted in the case of snap beans. Neither the processed peas nor snap beans gave a positive catalase test, Thompson (1942).

The greatest loss (18.7 per cent) of ascorbic acid in peas occurred during blanching with hard water. This water had a pH of 8.6 after the blanching was complete, which would be expected to favor oxidation of ascorbic acid.

The calcium content of the vegetables blanched in hard water was higher than in the samples blanched in the other waters, a result attributed to the large amount of calcium in the blanch water, and in confirmation of the results of Horner (1936).

Certain irregularities in the cooked samples can be explained, partly at least, by the increase in total solids during cooking. Water is apparently forced out during the cooking process. In general, the cooked samples showed slightly lower contents of water-soluble vitamins. This is to be expected because of the leaching effect of the boiling water.

SUMMARY

Greater retention of vitamin C by the blanching methods described was noted when compared with standard commercial methods. However, this had changed between the time of blanching and end of the six months' storage period, with the final result that peas packed by home or commercial methods contained substantially the same amounts of this vitamin.

Vegetables blanched in hard water showed significant increases in calcium.

With this exception, the use of different types of potable water for blanching resulted in no significant differences in vitamin or mineral content of the blanched, stored, or cooked peas and snap beans.

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NUTRITIVE VALUE OF CANNED FOODS. VII. EFFECT OF COMMERCIAL CANNING AND SHORT-TIME STORAGE ON ASCORBIC ACID CONTENT OF GRAPEFRUIT JUICE[1]

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From a nutritional viewpoint the value of canned grapefruit juice is largely related to ascorbic acid content. Consequently the preservation of this factor is of the utmost importance to both the canner and the consumer of this product. Floyd and Fraps (1942) have reported a group of studies on the ascorbic acid content of fresh and canned grapefruit juices from the Rio Grande Valley of Texas. These investigators compared the average ascorbic acid content of 109 samples of canned grapefruit juice with the average content of 21 samples of grapefruit juice expressed from orchard fruit. While the raw and canned juices were not operationally related, the authors concluded that the average loss of ascorbic acid in commercial grapefruit-juice canning operations was 18.2 per cent.

More recently in an extensive survey of Florida grapefruit juice canneries, Moore, Wiederhold, Atkins, and MacDowell (1944) found that the average loss of ascorbic acid in the manufacture of canned grapefruit juice was only three per cent. In these experiments juice sampled at several points in the canning line was analyzed, the first sample being taken from the freshly extracted juice and the last from the canned product. By proper adjustment of sampling times, they were able to sample a given lot of juice wherever desired without interrupting the rate of flow through the cannery. As the result of a similar type of study, Ross (1944) has reported that freshly canned grapefruit juice retains 92 to 98 per cent of the original ascorbic acid content.

A detailed survey of the ascorbic acid retention in grapefruit juice during its preparation and canning was conducted in the Rio Grande Valley to obtain more information on this subject.

EXPERIMENTAL PROCEDURE

Through the co-operation of the Texas Canners' Association arrangements were made with the operators of canneries to carry out these studies in 12 plants. The operations during one day were surveyed at each of 10 plants. At the other two plants (VI and XI) the study was continued for an additional two days in order to check on day-to-day variations.

Information on the source and history of the particular fruit canned, the equipment used, and the canning operations was obtained at each plant.

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The time required for each step in the canning operation was determined to insure representative sampling in each lot of juice. Juice from selected lots of fruit was then sampled at predetermined points in the canning line.

Samples were taken consecutively (1) from the freshly extracted juice, (2) after screening, (3) at the holding tank outlet whenever possible, (4) from the pasteurized juice at the filler bowl, and (5) from the cooled cans. In some instances the arrangement of the canning line did not permit taking a sample after holding before the juice entered the pasteurizers. Additional sampling stations were necessary at Plant I which used deaeration and at Plant III which used a small heated holding tank for pasteurized juice (Table 1).

Both "step-by-step" runs and "over-all" runs were made. In the step-by-step runs samples were taken at every sampling station. In the first part of the survey over-all runs were made by sampling the freshly extracted juice and the final canned product. Later, for reasons explained under sampling procedure, the first sample for over-all runs was taken after screening.

Two sampling procedures were used. In Procedure A samples were prepared by measuring 10-ml. portions of stabilizing solution (three per cent metaphosphoric acid and eight per cent acetic acid in copper-free water) into 50-ml. Erlenmeyer flasks. Ten ml. of collected juice was then pipetted into each flask. Aliquots for titration were drawn from these preparations. Four or five such samples were taken at each station over a 10-minute period. For sampling Procedure B, 50 ml. of stabilizing solution were measured into a 100-ml. volumetric flask. Samples of approximately five ml. were taken once a minute from the line for 10 minutes so that the flask was filled to the mark. Aliquots were taken from the mixed contents of the flask for titration. This procedure permitted a larger number of samplings in each run, is simpler, and seems less subject to error than Method A. For analysis of the canned product six of the cooled cans were collected from each lot of juice over a 10-minute period. These were analyzed on the same day as packed. The cans were shaken thoroughly, opened, and sampled immediately. Under sampling Procedure A, a separate sample was prepared from each can. Under Procedure B, aliquots of approximately eight and one-third ml. were pipetted from each can into the sampling flask so as to fill it to the mark.

The freshly extracted juice was prepared for the above sampling procedures by filtering through coarse gauze. It was noticed after a few runs that the screened juice tended to have a higher ascorbic acid content than the freshly extracted juice prepared in this manner. This seems to be due to the fact that the screened juice contains more pulp than the filtered samples. Since this pulp or "rag" has a higher ascorbic acid content than the juice alone, the ascorbic acid content of the screened juice will vary with its pulp content. For this reason it was felt that the screened juice was more representative of the canned product than the freshly extracted juice. Therefore, the later over-all runs were made by sampling the screened juice and the canned juice. The per cent retention of ascorbic acid during canning has been calculated on the basis of the content of the

screened sample as much as possible. Retention of ascorbic acid was calculated on the basis of the freshly extracted juice wherever the screened juice was not sampled. For the sake of uniformity, the latter figures (in parentheses in Table 1) have been excluded from the averages. All samples have been analyzed by titration with 0.02 per cent aqueous sodium-2,6-dichlorobenzenoneindophenol solution which was standardized daily against crystalline ascorbic acid (Merck).

In addition to the six cans of juice that were analyzed immediately after canning, six more were collected from each step-by-step run and stored for 40 days at room temperature of 18.3 to 26.7°C.(65 to 80°F.). At the end of the storage period measurements of headspace, vacuum, Brix, and titratable acidity were made. Aliquots were taken according to Procedure B and analyzed for ascorbic acid.

RESULTS AND DISCUSSION

The average retention of ascorbic acid during canning operations for all plants was 96.5 per cent (Table 1). This value checks very well with the figure (97 per cent) reported by Moore *et al.* (1944). Average retention for individual plants ranged from 92.2 per cent for Plant III to 99.8 per cent for VI (first day's operations). The daily average retentions for Plants VI and XI are quite uniform (98.7, 97.8, and 96.9 per cent for Plant VI; 97.2, 96.0, and 97.1 per cent for Plant XI). The low retention in Plant III appears to be due to the practice of holding the hot juice after pasteurization. The line arrangement of this plant did not permit the taking of samples immediately before pasteurizing, but the small losses incurred in holding tanks before pasteurization at other plants indicate that the loss occurs in the heated juice. With the exception of this case, the magnitude of the losses of ascorbic acid during canning did not seem great enough to justify any attempt to correlate them with differences in the canning equipment.

The retention of ascorbic acid during the storage period was calculated on the basis of the amounts found immediately after canning and at the end of the storage period. Individual samples retained from 83 to 100 per cent of their ascorbic acid. The average retention for all stored samples was 91.3 per cent. The differences in retention could not be definitely correlated with Brix, titratable acidity, headspace, or vacuum. The upper part of the range compares with values reported by Ross (1944) on the retention of ascorbic acid in canned, unsweetened grapefruit juice after one month's storage at room temperature. More complete work on the effect of storage at various temperatures on the ascorbic acid content of grapefruit juice is being carried out elsewhere in the nutrition program of which this study is a part.

SUMMARY

The effect of commercial canning practice on the ascorbic acid content of grapefruit juice has been studied in 12 canneries of the Rio Grande Valley. The retention of ascorbic acid during canning ranged from 92.2 to 99.6 per cent with an average retention for all plants of 96.7 per cent. During subsequent storage for 40 days (average about 72°F.) samples of

TABLE 1

Ascorbic Acid Content and Retention During Canning and After 40-Day Storage of Grapefruit Juice From Rio Grande Valley Canning Plants

Plant and runs	Freshly extracted	After screening	From holding tank		From filler bowl	Canned juice immediately after cooling		Sampling method	Stored 40 days	
						Ascorbic acid	Retained		Ascorbic acid	Retained[1]
DEAERATOR										
	mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*pct.*		*mg./100 ml.*	*pct.*
I—1	39.7	40.0	40.6	38.9	41.4	40.8	102.0	A	38.4	94.0
2	44.7	42.9	42.9	41.3	43.4	41.5	96.8		40.6	98.0
Av.	42.2	41.4	41.7	40.1	42.4	41.1	99.4		39.5	96.0
II—1	47.1	51.0	49.1	.....	49.5	53.0	103.9	A	47.5	89.5
2	(48.0)	.....	.....	.....	.....	(50.4)	(105.0)		.....	.....
3	50.4	51.6	51.3	.....	45.0	46.4	90.0		44.9	96.8
Av.	48.7	51.3	50.5	.....	47.2	49.7	96.9		46.2	93.1
PASTEURIZED[2]										
III—1	43.7	46.0	.....	40.7	41.0	42.3	91.9	A	.....	.....
2	41.3	42.8	.....	41.0	39.2	39.4	92.1		37.2	94.5
3	43.5	45.5	.....	43.2	43.1	42.4	92.7		38.9	91.8
Av.	42.8	44.8	.....	41.6	41.1	41.3	92.2		38.0	93.1
IV—1	42.9	43.2	43.2	.....	40.4	39.8	92.1	A	36.8	92.5
2	(42.6)	.....	.....	.....	.....	(41.3)	(96.8)		.....	.....
3	(40.0)	.....	.....	.....	.....	(40.3)	(99.3)		.....	.....
4	42.4	43.1	41.7	.....	42.5	41.1	95.4		39.8	97.0
Av.	42.6	43.1	42.4	.....	41.4	40.4	93.7		38.3	94.7
V—1	38.5	39.4	39.3	.....	36.5	36.9	92.6	A	35.7	96.8
2	(35.9)	.....	.....	.....	.....	(35.7)	(95.5)		.....	.....
3	36.9	38.0	38.2	.....	36.5	36.5	96.1		35.6	97.6
Av.	37.7	38.7	38.7	.....	36.5	36.7	94.3		35.6	97.2
VI—1	37.9	37.6	.....	.....	37.7	37.5	99.7	A	37.2	99.3
2	(37.6)	.....	.....	.....	.....	(37.5)	(99.7)		.....	.....
(a) 3	(38.7)	.....	.....	.....	.....	(37.0)	(95.4)		.....	.....
4	36.4	36.5	.....	.....	36.5	36.4	100.0		36.4	100.0
Av.	37.1	37.0	.....	.....	37.1	36.9	99.8		36.8	99.6
VI—1	38.6	38.4	.....	.....	37.6	37.7	98.0	B	32.1	85.2

VI—1	38.6	38.4	.....	.....	37.6	37.7	98.0	B	32.1	85.2
2	40.3	38.4	.....	.....	39.7	37.3	96.3		33.6	90.0
(b) 3	.....	37.7	.....	.....	.....	36.5	96.9		.....	.....
4	.....	41.3	.....	.....	.....	40.3	97.6		.....	.....
5	.....	40.5	.....	.....	.....	39.0	96.3		.....	.....
Av.	38.2	38.7	.....	.....	37.9	37.7	97.8		32.8	87.6
VI—1	.....	39.3	.....	.....	.....	36.2	92.1	B	.....	.....
2	.....	37.1	.....	.....	.....	37.4	100.7		.....	.....
(c) 3	.....	40.2	.....	.....	.....	38.5	95.8		.....	.....
4	41.2	41.4	.....	.....	40.8	40.6	98.1		34.7	85.3
5	39.2	39.6	.....	.....	38.7	38.6	97.6		37.2	96.5
Av.	40.2	39.5	.....	.....	39.1	38.2	96.9		35.9	90.9
VII—1	40.1	39.9	41.1	.....	39.8	39.8	99.2	B	35.2	88.5
2	(42.5)	.....	.....	.....	.....	(41.7)	(98.1)		.....	.....
3	(41.0)	.....	.....	.....	.....	(39.4)	(96.0)		.....	.....
4	(40.5)	.....	.....	.....	.....	(38.8)	(95.8)		.....	.....
5	39.4	39.1	38.3	.....	37.0	36.9	94.2		32.3	87.6
Av.	39.7	39.5	39.7	.....	38.4	38.3	96.7		33.7	88.0
VIII—1	39.3	39.6	39.4	.....	37.8	37.3	94.2	B	33.3	89.3
2	39.3	40.4	40.7	.....	39.0	38.9	96.3		34.3	88.2
3	40.0	41.7	42.9	.....	40.1	40.3	96.6		.....	.....
4	.....	43.1	.....	.....	.....	41.6	96.5		.....	.....
5	.....	43.0	.....	.....	.....	41.5	96.5		.....	.....
Av.	39.5	41.5	41.0	.....	39.0	40.1	96.0		33.8	88.7
IX—1	38.0	37.8	.....	.....	36.2	36.4	96.3	B	32.9	90.5
2	37.3	38.4	.....	.....	37.9	37.7	98.2		33.3	88.4
3	.....	40.7	.....	.....	.....	40.4	99.2		.....	.....
4	.....	37.7	.....	.....	.....	36.2	97.6		.....	.....
5	.....	38.1	.....	.....	.....	37.4	98.2		.....	.....
Av.	37.6	38.4	.....	.....	37.0	37.6	97.9		33.1	89.4
X—1	42.7	43.6	42.2	.....	41.5	40.5	92.9	B	36.4	89.8
2	43.0	42.4	40.8	.....	39.6	40.5	95.1		35.8	88.5
3	40.6	40.8	40.5	.....	39.3	39.1	95.8		.....	.....
4	.....	41.4	.....	.....	.....	40.5	97.8		.....	.....
5	.....	40.5	.....	.....	.....	40.4	99.7		.....	.....
Av.	42.1	41.8	41.2	.....	40.1	40.2	96.2		36.1	89.1

TABLE 1 *(Concluded)*

Plant and runs	Freshly extracted	After screening	From holding tank		From filler bowl	Canned juice immediately after cooling		Sampling method	Stored 40 days	
						Ascorbic acid	Retained		Ascorbic acid	Retained[1]
	mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*pct.*		*mg./100 ml.*	*pct.*
XI—1	.....	40.0	.....	.....	.....	38.7	96.7	B	.....	.....
2	.....	40.7	.....	.....	.....	36.0	88.5		.....	.....
(a) 3	38.5	38.0	.....	.....	38.0	38.0	100.0		35.3	93.0
4	.....	39.0	.....	.....	.....	38.0	97.5		.....	.....
5	39.4	39.7	.....	.....	38.6	41.1	103.5		33.7	83.0
Av.	38.9	39.5	.....	.....	38.3	39.4	97.2		34.5	88.0
XI—1	40.2	40.9	.....	.....	39.6	39.0	95.3	B	35.5	89.7
2	.....	40.8	.....	.....	.....	39.1	95.5		.....	.....
(b) 3	.....	39.4	.....	.....	.....	37.8	96.0		.....	.....
4	.....	39.4	.....	.....	.....	38.4	97.5		.....	.....
5	39.5	39.8	.....	.....	39.3	38.0	95.5		34.7	94.0
Av.	39.8	40.1	.....	.....	39.4	38.5	96.0		35.1	91.8
XI—1	40.4	40.6	.....	.....	39.2	38.4	94.7	B	33.1	86.3
2	.....	40.5	.....	.....	.....	40.2	99.5		.....	.....
(c) 3	40.0	40.6	.....	.....	38.8	38.4	94.8		33.6	87.6
4	.....	40.3	.....	.....	.....	39.0	97.0		.....	.....
5	.....	39.0	.....	.....	.....	38.8	99.3		.....	.....
Av.	40.2	40.2	.....	.....	39.0	39.0	97.1		33.3	86.9
XII—1	.....	47.1	.....	.....	.....	44.7	95.0	B	.....	.....
2	42.2	43.1	42.8	.....	41.0	40.9	95.2		34.3	83.8
3	.....	41.7	.....	.....	.....	40.0	96.0		.....	.....
4	39.4	40.6	40.6	.....	39.4	39.2	96.7		35.0	89.5
Av.	40.8	43.1	41.7	.....	40.2	41.2	95.7		34.6	86.6
Average of all juices sampled[2]	40.7	40.9	.....	.....	39.7	39.4	96.5		36.0	91.3

NOTE: The juice going through the screening operation in Plant III represents a combination of the first extracted juice and a second extraction made on the recovered pulp with a screw extractor. Ascorbic acid content of second extract: mg./100 ml. = 47.5, 51.1, 51.0 (Av. 49.9).

[1] The per cent retentions during storage were calculated on the basis of the amounts in the canned juices immediately after canning and at the end of the storage period. [2] Held in heated tank after pasteurization. [3] Excepting individual values in parentheses.

grapefruit juice retained, on the average, 91.3 per cent of the ascorbic acid present in the freshly canned juice.

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PLAIN BUTTER CAKES AND BAKED CUSTARDS MADE FROM SPRAY-DRIED WHOLE-EGG POWDER[1,2]

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The rapid growth of the egg-drying industry since 1941 has stimulated interest in the cooking properties of spray-dried whole-egg powder. Prior to 1940, considerable quantities of dried white, yolk, and whole egg were used commercially. According to LeClerc and Bailey (1940), approximately 75 per cent of the whole-egg powder produced was used in bakery products, although some was used in noodles, food-beverage powders, prepared puddings, ice cream mixes, and mayonnaise. Few studies have been reported on the use of whole-egg powder in cooking.

In the work thus far reported, there has been some disagreement among investigators as to the quality of products obtained when dried eggs were used for cooking. Termohlen, Warren, and Warren (1938) reported that whole-egg powder could be used successfully in baked foods, but much less satisfactorily in scrambled eggs, custards, and other products in which egg was the main ingredient. LeClerc and Bailey (1940) found the use of dried-egg powder not satisfactory for cake baking. Bennion, Hawthorne, and Bate-Smith (1942) produced satisfactory sponge cakes from good-quality, spray-dried whole-egg powder, although the cakes were inferior to those made with fresh eggs. These workers also reported that the undesirable flavor of some egg powders which made them unsatisfactory for custards or scrambled eggs was not so objectionable in sponge cakes. Jordan and Sisson (1943b,c) reported that muffins and baked custards, rating as high as fresh-egg products, were made from good-quality, spray-dried whole-egg powder.

The variation in the results of the different workers may have been due in part to differences in the quality of the egg powders used. Among the factors affecting quality are methods of processing and conditions of storage. In a progress report on the effect of storage time and temperature on the cooking quality and flavor of whole-egg powder Shank (1942) reported that baked custards made from egg powder which had aged considerably were not firm and had crusts that were thick and dark yellow in color; she found that the off-flavor of egg powder owing to improper storage was less evident in plain cakes than in scrambled eggs, popovers,

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[2] This paper is based upon part of the thesis presented by Jean E. Ary in partial fulfillment of the requirements for the degree of Master of Science in Home Economics at Purdue University, February, 1944.

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mayonnaise, or baked custards; the volume of cakes was poor only when prepared from egg powders which had developed disagreeable flavors. Woodcock and Reid (1943) and Thistle, White, Reid, and Woodcock (1944) used test sponge cakes for evaluating the effect of different treatments upon the quality of dried whole-egg powder.

Among the first of the physical and chemical tests developed for evaluating the quality of egg powders for cooking was the solubility index. Stuart, Grewe, and Dicks (1942) found a positive correlation between the solubility index of spray-dried whole-egg powders and the volume and quality of pound cakes, and also between solubility and the type of coagulum formed in scrambled eggs. These workers discussed two types of insolubility, one caused by changes in the egg owing to processing, another caused by changes resulting from storage. More recently, McNally and Dizikes (1944) made the statement that measurements of solubility have been correlated with uses of dried eggs in custards and in baking, and that dried eggs of low solubility produce weak custards and heavy cakes.

Just before the studies herein reported were undertaken, the methods of Stuart, Grewe, and Dicks (1942) for determining the solubility index of dried whole eggs were published. The two methods described by these investigators were for the determination of the "centrifuged heat coagulable solubility index" and the "Esbach's sedimentation solubility index." Later, Stewart, Best, and Lowe (1943) reported a refractometric method for determining the solubility of fat-free dried egg. Other methods, several of them involving the use of the refractometer, have since been suggested. Hawthorne (1944) has summarized and evaluated some of these methods.

The main purpose of this study was to compare the effects of spray-dried whole-egg powders differing in quality, as measured by the Esbach's sedimentation solubility index, upon the properties of plain butter cakes and baked custards. Before undertaking this part of the work, a preliminary study was made to determine the effect of two different methods of incorporating the egg powder into cake batters. Throughout the study, 13.5 grams of spray-dried whole-egg powder plus 34.5 grams of water were used as being equivalent to one 48-gram, fresh, whole egg, Jordan and Sisson (1943a).

I. PLAIN BUTTER CAKES

EXPERIMENTAL PROCEDURE

Series I. In this series three groups of cakes were made. In one group, the dried whole-egg powder was sifted with the flour and baking powder and the water necessary for rehydration was added to the milk; in a second group, the egg powder was reconstituted, then added in the same way as fresh egg; in the third group of cakes, fresh eggs were used.

The egg powder, freshly dried, was obtained from a commercial drying plant and was kept under refrigeration [temperature about 5°C.(41°F.)] from the time it was received in the laboratory until it was used. This series of cakes was baked within a two-month period after the receipt of the egg powder. The fresh eggs were obtained as needed from the Depart-

ment of Poultry Husbandry of Purdue University. Uniformity in quality and quantity of ingredients and such other factors as temperature of ingredients, method of mixing, and time and temperature of baking were controlled as far as possible. In each baking period three cakes—one from each variant—were made, but the order of mixing the cakes and the oven used for baking were rotated from period to period by a prearranged plan in order to eliminate effects owing to these factors.

The formula used was that of Lowe and Nelson (1939) modified by reducing the salt from six to two grams. Half of the recipe was used for each cake mix. Two small loaf cakes were baked from each mix; 235 grams of batter were weighed into each pan. The cakes, mixed by hand, were prepared by Lowe's (1937) conventional method, modified as to the total number and distribution of strokes used for mixing.

The determinations included the weight of the baked cake; the volume, measured by seed displacement [Cathcart and Cole (1938)]; the area of a slice of cake (or index to volume) measured by a planimeter [Platt and Kratz (1933)]; the water absorbing ability on the day of baking and the day following baking [Swartz (1938)]; and quality as judged by scores given by a palatability committee of six persons.

The palatability judges were asked to check whether the cake was made from fresh or dried egg, or to indicate if they could not tell which form of egg was used.

Series II. Five spray-dried whole-egg powders, designated as A, B, C, D, and E, were used in this series of cakes. All except C had been dried in a Mojonnier type of drier; C in a room-type drier. Powder A was the same as used in cake Series I, but had been stored in the refrigerator 10 months; Powder B had been of good initial quality, but had been held in storage in a barrel in a warehouse (temperature not controlled) for a period of 12 months, then removed to the laboratory and held in a refrigerator for seven months; Powders C, D, and E had been held in the refrigerator eight months before being used in this series of cakes. Powder C was not designated by grade; Powder D was marked Grade A; Powder E, Grade B.

The solubility index, measured by the Esbach's sedimentation test [Stuart, Grewe and Dicks (1942)], of each of the powders was determined at the beginning and end of this series of bakings.

For this series of cakes, the egg powder was incorporated into the batter by sifting it with the flour and baking powder and the water for rehydration was added to the milk. The formula, method of mixing, time and temperature of baking, and the objective and subjective measurements made on the baked cakes were the same as for cake Series I. Five cake mixes—one from each egg powder—were baked each day; but the order in which the cakes were mixed and the oven used for baking were varied from day to day according to a prearranged plan.

RESULTS AND DISCUSSION

From both the subjective and objective measurements in cake Series I (Table 1) it was found that butter cakes similar to those made with fresh eggs can be made with good-quality, spray-dried whole-egg powder. The

judges who scored the cakes for palatability were unable to distinguish consistently between cakes made with fresh and dried eggs. The data (Table 1) also reveal that under the conditions of this experiment there was no significant difference in the quality of the cakes made by sifting the egg powder with the flour and baking powder and by reconstituting the dried egg before incorporating it into the batter. The method of sifting the egg powder with the flour has a distinct advantage in the ease and speed with which a cake can be mixed, and since the results are as satisfactory, this method of mixing can be recommended. The results of some further work indicate that this method of mixing is also satisfactory for plain cakes made with fats [4] other than butter.

TABLE 1

Averages of Judges' Scores and of Objective Measurements Made on Plain Butter Cakes Prepared From Spray-Dried Whole-Egg Powder and From Fresh Eggs

(12 cakes in each group)

Scores and measurements	Kind of egg and treatment			Difference in means necessary for significance at:	
	Fresh egg	Dried egg		5-per cent level	1-per cent level
		Sifted	Reconstituted		
Judges' scores[1]:					
Grain	13.3	13.7	13.1	0.69	0.92
Crumb	13.3	13.2	13.0	0.57	0.78
Tenderness	14.1	13.9	14.0	0.42	0.55
Velvetiness	13.7	13.7	13.5	0.45	0.60
Flavor	14.0	14.1	13.8	0.74	0.88
Color[2]	14.8	14.8	14.8	.....	.....
Eating quality	13.9	13.9	13.8	0.51	0.78
Total of unweighted scores	97.1	97.3	96.0	.....	.....
Objective measurements:					
Weight (gm.)	195.5	196.4	197.2	.....	.....
Volume (c.c.)	489	485	494	20.84	27.81
Volume per 100 grams (c.c.)	250	247	250	.....	.....
Index to volume by planimeter (sq. in.)	6.40	6.35	6.51	0.22	0.29
Water absorbing ability:					
Day of baking (gm.)	11.5	11.6	11.5	0.54	0.72
Day after baking (gm.)	10.6	10.8	10.9	0.59	0.79

[1] Possible score for each factor, 15; total 105. [2] Under the factor of color, uniformity and evenness in color and freedom from any yellow specks were stressed.

A summary of the data on cakes made with the five egg powders, A, B, C, D, and E, used in cake Series II (Table 2) indicates that the cakes from Powder D were in all respects the best. The palatability judges, not having been informed that all the cakes in this series were made from dried eggs, frequently indicated that they thought cakes from Powder D were made from fresh eggs. The cakes from Powders B and E were

[4] In a short study it was found that even with cakes made with lard as the fat, the method of sifting the egg powder with flour gave as good results as the use of the conventional-sponge method of mixing, Lowe and Nelson (1939), which was developed especially for lard cakes.

smaller in volume and less desirable in flavor and eating quality than cakes made from Powders A, C, and D, especially D; however, the cakes from B and E powders were not judged to be extremely poor.

TABLE 2

Averages of Judges' Scores and of Objective Measurements Made on Plain Butter Cakes Prepared From Five Samples of Spray-Dried Whole-Egg Powder

(Five cakes in each group)

Scores and measurements	Egg powder used in cakes					Difference necessary for significance at:	
	A	B	C	D	E	5-per cent level	1-per cent level
Judges' scores[1]:							
Grain	13.0	11.8	12.7	12.8	11.2	0.79	1.07
Crumb	12.8	12.3	12.5	13.5	12.7	0.86	1.18
Tenderness	13.4	13.3	13.4	14.0	13.2	0.51	0.69
Velvetiness	13.2	12.6	12.8	13.4	12.6	0.71	0.96
Flavor	13.4	11.1	12.6	13.8	11.8	1.40	1.90
Color	14.4	14.1	14.2	14.5	12.9	0.76	1.03
Eating quality	13.1	11.5	12.6	13.7	11.7	1.01	1.38
Total of unweighted scores	93.3	86.7	90.8	95.7	86.1	4.43	6.05
Objective measurements:							
Weight (gm.)	200	203	201	201	202	.....	.....
Volume (c.c.)	465	455	463	480	447	10.54	14.38
Volume per 100 grams (c.c.)	233	224	230	239	221	.....	.....
Index to volume by planimeter (sq. in.)	6.36	6.20	6.39	6.45	6.16	0.23	0.31
Water-absorbing ability:							
Day of baking (gm.)	11.1	9.7	10.9	11.2	10.1	0.46	0.63
Day after baking (gm.)	10.5	9.5	10.7	10.7	9.9	0.70	0.96

[1] Possible score for each factor 15; for total 105.

The solubility indices of Powders B and E were 2.7 and 3.3, respectively, contrasted with indices of 4.6, 4.6, and 4.7 for Powders A, C, and D. Assuming that the solubility index is an indication of quality of the egg powder, it appears that powders of lower solubility (and lower quality) tend to yield cakes of lower volume and poorer eating quality.

II. BAKED CUSTARDS

EXPERIMENTAL PROCEDURE

The same five egg powders used in cake Series II were used in making baked custards. Since the custard series was prepared before Series II of the cakes, the egg powders had not been in storage for so long a period. At the time of making the custard study, Powder A had been stored in the refrigerator [temperature about 5°C.(41°F.)] for four months, Powders C, D, and E for two months, and Powder B for one month after being brought from the warehouse where it had been in storage for 12 months. Ten bakings of custards were made from each powder and also from fresh eggs; each kind of egg was used in each of the 10 baking periods.

Preliminary work indicated that for Powders B and E, the usual formula of one egg to one cup of milk would not make custards firm enough for the satisfactory use of the penetrometer for measuring firmness; hence the proportion of egg to milk was doubled for this series of custards. The formula as used was:

Milk, 237 milliliters
Sugar, 25 grams
Egg, fresh, 96 grams or
Eggs, dried, 27 grams and 69 milliliters of water.

The milk was heated to 80° C. (176° F.), added to the reconstituted egg (or fresh egg) and sugar, and the mixture was thoroughly stirred. Measured amounts of each custard mix were poured into two glass custard cups. The duplicate cups were placed in a pan of hot water and the custards baked to an internal temperature of 88°C.(190.4°F.) in an oven maintained at 176.6°C.(350°F.). The plan for baking the 60 custard mixes provided for rotation of the order of mixing and of position in the ovens during baking. The pH of the milk used each day and of each uncooked custard mix was determined on a Beckman pH meter with glass electrode. A record was kept of the temperature rise in one of each of the duplicate cups of custard during the baking period.

The baked custards were cooled to room temperature, covered, and stored overnight in a refrigerator. The following day the crusts were carefully removed and penetrometer readings taken. The custards were then removed from the cups and examined organoleptically.

The statistical treatment of the data included an analysis of variance [5] of the penetrometer readings.

RESULTS AND DISCUSSION

The average penetrometer readings for the baked custards, the pH values of the uncooked mixes, the solubility indices of the egg powders, and the average time required for the custards to reach an internal temperature of 88°C.(190.4°F.) are given (Table 3). Since the lower the penetrometer reading the firmer the custard, it is evident that egg Powders A, C, and D produced custards similar in firmness and that the custards from these powders were much firmer than those made from Powders B and E, and even firmer than the custards from the fresh eggs. This latter observation is not in agreement with the findings of Jordan and Sisson (1943c) in a study in which they found that fresh eggs produced custards of greater firmness than dried eggs when the formula used the proportion of one egg (48 grams fresh egg or 13.8 grams dried egg plus 34.2 milliliters of water) to one cup (237 milliliters) of milk. Since in the study herewith reported the amount of egg used with one cup of milk was doubled, any error in the estimation of dried-egg powder equivalent to the solids in fresh egg would be increased. Other factors, such as variability in the quality of the fresh eggs and of the egg powder, may have influenced the results.

[5] Acknowledgment is made to Professor S. R. Miles of the Department of Agronomy of the Purdue Agricultural Experiment Station for help with the analysis of variance.

The custards from Powders B and E had lower pH values, took longer time to reach 88°C., and were less firm than custards made from Powders A, C, and D and from fresh eggs. The solubility indices of Powders B and E were markedly lower than for Powders A, C, and D. Hence, lower solubility indices seem to be associated with less firm custards and indicate lower thickening or coagulation properties of the egg powder.

TABLE 3

Average Penetrometer Readings, pH Values, Baking Times for Custards, and Solubility Indices of Five Spray-Dried Whole-Egg Powders

Egg powder	Penetrometer readings[1] for custards (0.1 mm. units)	pH of uncooked custard mixes	Baking time to reach internal temperature of 88°C. (190.4°F.)[2]	Esbach's sedimentation solubility index of egg powder[3]
			min.	*ml.*
A	212.4	7.3	34.8	4.6
B	293.2	6.9	42.7	2.7
C	212.3	7.3	33.7	4.6
D	212.7	7.2	34.0	4.6
E	230.5	7.1	43.1	2.8
Fresh egg	222.8	7.1	33.1	....

[1] Difference in penetrometer readings necessary for significance at five-per cent level, 5.99; at one-per cent level, 8.04. [2] Difference in time of baking necessary for significance at five-per cent level, 2.64 minutes; at one-per cent level, 3.52 minutes. [3] Values of solubility indices represent averages of determinations made immediately before and immediately after the series of custards was prepared.

An analysis of variance of the firmness of the custards, measured by the penetrometer, in which the factors of the day of baking, the ovens in which the custards were baked, and the egg powders (or fresh egg) were considered, showed that the effect of the ovens was negligible and that there was a significant variation in the firmness of the custards from day to day. However, this variation was small as compared with the great variance owing to the difference in the egg powders.

The baking time was significantly greater for the custards made from Powders B and E than for the other custards; the time-temperature relations during baking are shown (Fig. 1). The rate of rise was very similar for all the custards during the first part of the baking period or until the temperature of 68 to 70°C. (about 154 to 158°F.), at which coagulation of egg protein begins, was reached. From that point on, the temperature rise in custards from Powders B and E was much slower than for the other custards. The B and E custards were characterized by thick, dull crusts and by streaks of minute yellow particles throughout the gel. It may be possible that one factor in the slowing down of heat penetration in the B and E custards was the interference to heat conduction offered by the numerous, small particles of insoluble egg powder. Until the temperature at which coagulation begins was reached, heat was probably transferred by convection and conduction. As gelation progressed, the heat transfer was probably mostly by conduction and the numerous small particles in the low solubility powders probably offered interference to the conduction of heat. Carr and Trout (1942) reported heat penetration

slower in custards made with homogenized than with unhomogenized milk.

Organoleptic tests on relative firmness of the custards agreed with the penetrometer readings. In flavor, the fresh-egg custards ranked first, D, A, and C custards next in order, and B and E were undesirable in flavor.

Low solubility in egg powder—indicative of loss in quality, whether due to long storage as in Powder B, or to poor initial quality as in Powder E—seems to indicate poor thickening properties.

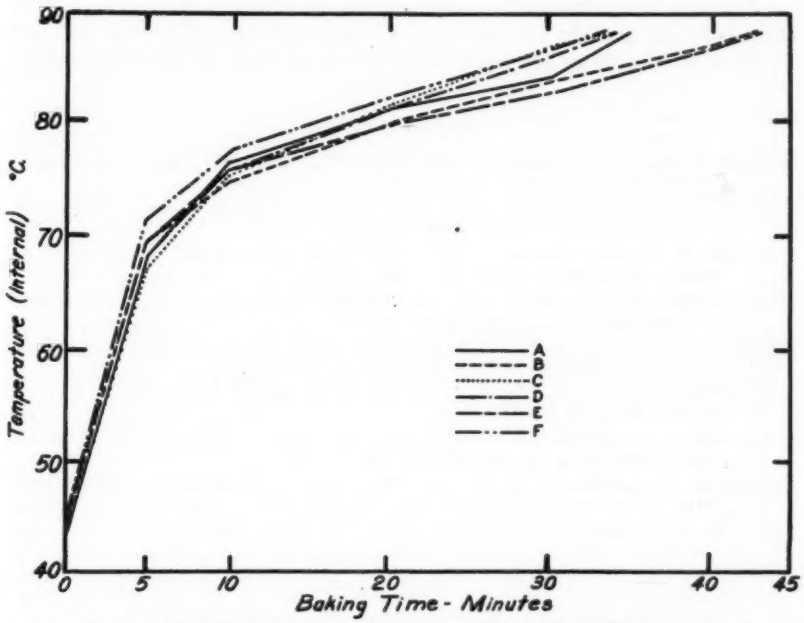

FIG. 1. Time-temperature relations during the baking of custards from fresh egg, F, and from five dried-egg powders, A, B, C, D, and E.

SUMMARY AND CONCLUSIONS

Two methods of incorporating whole-egg powder into plain cake batter were studied, and the relation of the solubility indices of five spray-dried whole-egg powders to the relative quality of plain butter cakes and to the firmness of baked custards was determined.

Under the conditions of this experiment, no significant differences were observed in the volume, grain, flavor, and general desirability of cakes prepared with fresh eggs or with good-quality whole-egg powder which was incorporated into the cake batter either by reconstituting the egg or by sifting the powder with the flour and baking powder.

The two egg powders having the lower solubility indices produced cakes of less volume and poorer eating quality, and custards which were less firm and less desirable in flavor than the three powders having the higher solubility indices.

The rate of heat penetration in baked custards made with the low-solubility egg powders was significantly slower than in custards made with fresh eggs or with the three powders having the higher solubility indices.

The poorer flavor of the two egg powders of low-solubility indices was more noticeable and more objectionable in custards than in the plain cakes.

Plain butter cakes and baked custards comparable in desirability to similar products made with fresh eggs can be made with good-quality, spray-dried whole-egg powders.

A simple and time-saving mixing procedure which yields satisfactory cakes is to sift the egg powder with the flour and baking powder and add to the milk the water necessary for the rehydration of the egg powder.

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OBSERVATIONS ON THIAMIN CONTENT[1] OF STORED WHEAT

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Investigations dealing with the effect of storage on the thiamin content of wheat have been limited, owing in part to the assumption that this vitamin is stable during storage and to the difficulty of obtaining suitable samples. As wheat is kept in storage under various conditions and for considerable periods of time, information concerning the effect of this storage upon the thiamin content should be of interest. It is hoped that the results reported at this time will arouse interest and therefore lead to further work.

The thiamin content of wheat has been shown to be influenced by variety, environment, and location. Connor and Straub (1941) found from their work with wheat that the thiamin content appeared to be dependent on variety, protein content, and the environmental conditions under which it was grown. Applying the analysis of variance to their data obtained from winter wheat, Nordgren and Andrews (1941) showed that the effect of both variety and environment was highly significant in affecting the thiamin content. From a comprehensive study of winter wheats for two years, O'Donnell (1943) showed that the thiamin content of wheat was definitely influenced by season, variety, and location.

MATERIALS AND METHODS

The samples used in this study varied in age from five months to 51 years. The very old samples are principally of historical interest.

The method used for determining thiamin was essentially the thiochrome method as proposed by Hennessy (1942). All values listed in the tables are the average of duplicate determinations, and the precision of the determination was five per cent. Unless indicated otherwise, all thiamin results are given in micrograms per gram converted to a 15-per cent moisture basis. The moisture values shown represent the moisture content of the samples when ground for the thiamin determination.

RESULTS AND DISCUSSION

Samples of 1941 Kansas Tenmarq wheat which was grown in one field and stored under normal and abnormal conditions were available for this study. Part of the wheat was binned directly from the combine at a moisture content of approximately 17 per cent, while the remainder was binned at a normal moisture content of about 12.5 per cent. The wheat was

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stored in steel bins and was also used in an investigation designed to study the effect of ethylene gas on wheat binned at high and normal moisture contents. A complete history of the grain has been previously published by Hale, Schwimmer, and Bayfield (1943). The high-moisture wheat, as might be expected, heated and eventually went out of condition (spoiled) in spite of being turned several times. Samples were removed periodically from both the treated and untreated bins over a period of five months and held in cold storage until the thiamin determinations were made.

TABLE 1

Thiamin and Moisture Content of Wheat From Storage Experiment, 1941 Crop

Date	High natural moisture				Normal moisture			
	Bin 6—untreated		Bin 7—ethylene treated		Control[1]—untreated		Bin 8—ethylene treated	
	Thiamin	Moisture	Thiamin	Moisture	Thiamin	Moisture	Thiamin	Moisture
	μg./gm.	*pct.*	*μg./gm.*	*pct.*	*μg./gm.*	*pct.*	*μg./gm.*	*pct.*
June 23	3.39	17.1	3.45	17.2	3.11	12.5	.....	.....
June 25	3.58	17.3	3.37	17.0	.....	.....	.....	.....
June 26	3.30	17.0	3.44	17.0	.....	.....	3.44	12.5
July 1	3.39	17.6	3.45	16.3	.....	.....	3.42	12.0
July 9	3.17	17.0	3.32	15.1	.....	.....	.....	.....
Sept. 3	2.68	14.6	3.08	15.4	2.71	12.0	3.40	11.2
Oct. 2	2.56	15.0	2.67	14.7	.....	.....	3.11	11.8
Nov. 17	2.36	14.9	2.46	13.7	.....	.....	3.05	10.0

[1]Approximately three bushels stored in a metal container.

The thiamin values obtained for the wheats from all bins showed a definite decrease in thiamin with increasing age (Table 1). The high-moisture wheat which was treated with ethylene gas showed slightly less loss of thiamin than the untreated wheat. The treated wheat was not as badly damaged during storage as the untreated bin of similar moisture. The data presented in this table show that for wheat stored with high moisture a loss of approximately one microgram of thiamin occurred over a period of five months. This appears to be a considerable loss in such a short interval of time, but even the normal moisture wheat lost 0.4 microgram during the same period, indicating an appreciable loss of thiamin during favorable storage conditions in metal bins built inside a stone building.

A similar storage experiment was conducted in 1942, and several samples were available from this experiment. Part of the wheat was combined and binned at a moisture content of 15 per cent, while the remainder was normal-moisture wheat of approximately 13 per cent. Thiamin determinations were made on these samples in order to observe if they showed trends similar to those found in the 1941 crop (Table 2).

The wheat in Bins 6 and 10 was considerably higher in thiamin content than the wheat in Bins 4 and 12. Although all bins were filled with Tenmarq wheat, the wheat in Bins 4 and 12 came from one field, while that in Bins 6 and 10 came from another on the same farm.

As indicated (Table 2), there was a significant decrease in thiamin content of wheat between the September and the April, 1943, samplings.

These data support the 1941 results; namely, that there is a loss of thiamin in the wheat during storage, particularly when the wheat is of high moisture content and goes out of condition.

TABLE 2

Thiamin and Moisture Content of Wheat From Storage Experiment, 1942 Crop

Wheat sampled	High natural moisture				Normal moisture			
	Bin 6—untreated		Bin 10—ethylene treated		Bin 4—untreated		Bin 12—ethylene treated	
	Thiamin	Moisture	Thiamin	Moisture	Thiamin	Moisture	Thiamin	Moisture
	μg./gm.	*pct.*	*μg./gm.*	*pct.*	*μg./gm.*	*pct.*	*μg./gm.*	*pct.*
Late September	4.29	14.7	4.25	14.2	3.56	13.2	3.61	13.0
December	2.63	13.9	3.07	14.7	2.50	12.2	2.83	12.8
April, 1943	2.51	12.1	2.47	11.7	2.17	11.8	2.13	12.5

TABLE 3

Thiamin Content[1] of Some Old Wheat Samples

Variety	Source	Year grown	Thiamin
			μg./gm.
Fulcaster	Ohio	1899	0.29
Fulcaster	Ohio	1930	2.58
Fulcaster	Ohio	1942	3.74
Poole	Ohio	1899	0.74
Poole	Ohio	1930	2.94
Poole	Ohio	1942	3.99
Marquis	Colorado	1921	1.96
Marquis	Colorado	1924	2.17
Marquis	Colorado	1927	2.57
Marquis	Colorado	1931	3.89
Kanred	Colorado	1921	2.95
Kanred	Colorado	1924	3.11
Kanred	Colorado	1929	3.16
Defiance	Colorado	1922	3.43
Defiance	Colorado	1927	2.54
Kubanka	Colorado	1921	3.32
Turkey	Kansas	1891	1.20
Tenmarq	Kansas	1930	1.64
Turkey	Kansas	1932	2.88[2]
Fulcaster	Kansas	1932	3.25[2]
Kanred	Kansas	1939	3.72
Marquis	Minnesota	1932	2.60[2]
Sonora	Idaho	1932	4.19[2]
Club	Washington	1932	2.81[2]

[1]Analyzed 1942. [2]As received moisture basis, insufficient sample for moisture determination.

The thiamin content of some samples of wheat ranging from three to 51 years old are presented (Table 3). These samples, of unknown moisture histories, were stored in bottles or other containers under normal laboratory conditions. In appearance they seemed sound, although the very old samples showed evidence of their age. As the original thiamin contents of these wheats were unknown, any comparisons must be based upon the average values of present crops. In view of the variable ori-

gins and ages of the samples in this table, it is evident that there was a considerable loss of thiamin during prolonged storage, and it is rather remarkable that the trends are so consistent.

SUMMARY

Thiamin content was determined on a number of wheats which were stored for periods ranging from five months to 51 years.

It is evident that there is a loss of thiamin in wheat even under normal storage conditions. This loss is much greater under abnormal storage conditions where factors such as high moisture with consequent high temperatures are involved.

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ASCORBIC ACID, CAROTENE, CHLOROPHYLL, RIBOFLAVIN, AND WATER CONTENT OF SUMMER SQUASHES[1]

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For a long time, perhaps almost since the time that the Indians showed the American colonists how to grow pumpkins in the cornfields, squashes have been used for food. While preferences for different varieties change with localities and persons, summer squashes are very extensively and quite frequently included in the bill of fare for two or three months in the summer. In spite of the rather general use of this vegetable, a review of the literature revealed very little information concerning its vitamin content and this study was therefore undertaken to accumulate such data.

SOURCE AND VARIETIES OF SQUASHES

In order to secure squashes typical of those grown in this locality, several persons were asked to supply them from their Victory gardens. The soil and fertilizers, the maturity of the squashes when picked, and the length of time which elapsed between the removal of the squash from the vine and its delivery to the laboratory doubtless varied; but the rainfall, temperature, and hours of sunshine were the same for all the Victory gardens. According to Gunness (1944) these were as follows: rainfall in June, 4.70 inches, in July, 3.88 inches, and in August, 4.33 inches; average temperature 66.3°F. for June, 72.9°F. for July, and 72.4°F. for August; and hours of bright sunshine 279 for June, 335 for July, and 301 for August.

Seven varieties of summer squashes were studied. While this group may not include all of the varieties grown in other parts of the country, it includes those grown to any extent in New England. Five of the seven varieties were *Cucurbita pepo* L. and two were *Cucurbita moschata* L. Botanists are inclined to consider all pepos as pumpkins. However, regardless of the scientific classification of these plants, the seedsmen, growers, marketmen, and ultimate consumers consider them as squashes and they will be designated as such in this discussion.

Cocozella Vegetable Marrow (*Cucurbita pepo* L.) is a long, cylindrical, nearly straight squash that has a dark green exterior with longitudinal white streaks. All of the tissues inside the skin were nearly pure white. Zucchini Grey (*Cucurbita pepo* L.) has much the same conformation as the Cocozella, but instead of white stripes the intense green color of the exterior is interspersed in some areas with sufficient small white spots to produce a sort of greyish color as is indicated by the name of the squash.

[1] Contribution No. 548, Massachusetts Agricultural Experiment Station.

Early White Bush Scallop (*Cucurbita pepo* L.) is shaped like a shallow bowl with characteristic scalloped edges. Both the exterior and interior tissues are white, but by comparison the exterior has a very slight creamy tinge. Early Summer Prolific Straightneck (*Cucurbita pepo* L.) is cylindrical with a gentle tapering or decreasing diameter from the blossom to the stem end. It is ordinarily about 12 inches long and has an intense yellow skin and cream-colored interior. Early Summer Crookneck (*Cucurbita pepo* L.) has long been very popular and extensively grown in this locality. It is cylindrical and tapers from the blossom to the stem end and, as the name indicates, there is a very definite curvature at the stem end. The exterior is of a deep yellow color and the inner tissues are cream-colored. Butternut (*Cucurbita moschata* L.) is a relatively new variety of squash in this locality. It is cylindrical with an enlarged bulb-like section at the blossom end. The skin is cream-colored and the interior is a deep orange color. Golden Cushaw (*Cucurbita moschata* L.) has been grown in this area on a commercial scale for only a few years. It is a long, cylindrical squash with a curved neck at the stem end and a bulb-like portion at the blossom end. The exterior is cream-colored and the interior has a very pronounced orange color.

ASSAY PROCEDURE

The ascorbic acid content of the raw summer squashes was determined by a modification of the Holmes, Tripp, Woelffer, and Satterfield (1939) method. Fifty grams of squash and 200 c.c. of five per cent metaphosphoric acid were thoroughly mixed in a Waring blendor. The mixture was filtered and repeatedly washed with five per cent metaphosphoric acid until 500 c.c. of filtrate were obtained. Approximately 50 c.c. of the filtrate was transferred to a 125-c.c. Erlenmeyer flask, one-half gram of 300-mesh Bentonite was added, the mixture was shaken vigorously for one minute, allowed to settle, and filtered. Ten-c.c. portions of the clear, colorless filtrate were titrated with a standard 2-6 dichlorophenolindophenol dye solution.

An adaptation of the Wall and Kelley (1943) method was used to assay both carotene and chlorophyll in the same sample. A 50-gm. sample of squash and 130 c.c. of acetone were placed in a Waring blendor and vigorously agitated for five minutes. The mixture was filtered, with suction, through a medium-sized sintered funnel (using a pad of shredded filter paper, if necessary, to obtain a perfectly clear solution). The pad was washed with small portions of acetone and the clear filtrate was diluted to 200 or 250 c.c. with acetone. Aliquots were read in a Coleman Universal spectrophotometer using the 610 wave length. The amount of chlorophyll in the sample was calculated by comparing the readings with a standard curve prepared with 5X chlorophyll (American Chlorophyll Co.).

For the carotene determination 250 c.c. (10 c.c. if the carotene content is high) were transferred to a Squibb separatory funnel, 50 c.c. of Skellysolve B and 50 c.c. of distilled water and five c.c. of three per cent sodium sulfate were added and brought into intimate contact by vigorous shaking for one minute. When separation was complete the water-acetone layer

was removed to another funnel and extracted with 25 c.c. of Skellysolve B by shaking for 30 seconds. As soon as separation was complete, the water-acetone layer was discarded and the Skellysolve extracts were combined and filtered through No. 41 Whatman filter paper which was covered with five grams of sodium sulfate. The paper and sodium sulfate were repeatedly washed with small portions of Skellysolve. Filtrate and washings were evaporated nearly to dryness on a steam bath, 20 c.c. of Skellysolve were added, and the mixture was passed through an adsorbent column, according to the Wall and Kelley (1943) method, made up to volume, and read in a Coleman Universal spectrophotometer.

The riboflavin content of the various raw squashes was determined by a combination of the procedures suggested by Hodson and Norris (1939), Ferrebee (1940), Karrer and Fritzsche (1935), Sullivan and Norris (1939), Connor and Straub (1941), and Kemmerer (1941). Fifty grams of squash were cut finely and thoroughly mixed with 50 c.c. of 0.25N sulfuric acid in a Waring blendor, transferred to an extraction flask, and extracted for an hour or more on a hot plate. After cooling, a sufficient quantity of a 6.5-per cent tribasic sodium phosphate solution to produce a pH of 6.5, 60 c.c. of acetone, and sufficient water to make 350 c.c. were added. Most of the squash tissue settled rapidly to the bottom of the flask. Seventy c.c. or more of the supernatant liquid were centrifuged for five to 10 minutes to yield 50 c.c. of supernatant liquid which was transferred to an amber 100-c.c. flask. Potassium permanganate and hydrogen peroxide were used to clarify and decolorize. Forty c.c. of acetone were then added and the mixture thoroughly agitated. When the flocculent precipitate separated, the mixture was vigorously shaken for 30 seconds, acetone was added to make 100-c.c. volume, and the mixture was filtered through No. 41 Whatman paper. Three aliquots, i.e., a sample and positive and negative controls, were prepared and read in a Coleman Universal spectrophotometer as described by Holmes, Jones, Wertz, and Kuzmeski (1943). The entire assay procedure was carried out in amber glass in a semidark room.

The chlorophyll, carotene, and riboflavin assays were made in a darkened laboratory described in detail by Holmes, Jones, Wertz, and Kuzmeski (1943). The light intensity was about three-foot candles and amber glassware was used. Thus destruction of carotene by chlorophyll in the presence of light, as described by Pepkowitz (1943, 1944), was greatly reduced if not eliminated.

The amount of water in the squashes was determined by the Association of Official Agricultural Chemists' (1940) method.

Since it is customary to include the skin and seeds of the immature squash when preparing it for the table, the assay samples were cut from the squash so that the skin, seeds, and interior tissues were included in proper proportion. The samples were disintegrated in the Waring blendor to such an extent that the fluid mixture could be removed with a pipette having a wide tip.

RESULTS

The results of the assays of 20 raw summer squashes for their ascorbic acid, carotene, chlorophyll, riboflavin, and water content are reported

(Table 1); the table also includes the name commonly used in this locality and information concerning the external color.

TABLE 1

Ascorbic Acid, Carotene, Chlorophyll, Riboflavin, and Water Content of Summer Squashes

Name and color	Ascorbic acid	Carotene	Chlorophyll	Riboflavin	Water
	mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
Cocozella Vegetable Marrow (green)	2.1	0.22	7.10	.161	96.05
	3.0	0.16	7.00	.184	95.84
	7.4	0.10	4.10	.175	95.36
Zucchini Grey (Green)	4.5	0.39	12.80	.....	95.33
	4.8	0.33	11.70	.....	95.31
Early White Bush Scallop (white)	11.0	0.05	0.55	.037	94.46
	13.7	0.06	0.65	.055	92.83
	25.5	0.06	0.45	.055	94.01
Early Summer Prolific Straightneck (yellow)	14.4	.....	0.38	.....	94.22
	11.9	.....	0.50	.....	94.29
	10.1	0.40	0.50	.....	93.54
Early Summer Crookneck (yellow)	11.3	0.40	1.25	.117	91.34
	9.9	0.40	1.50	.115	93.20
	12.3	0.20	1.10	.125	90.73
Butternut (cream)	10.0	3.46	0.68	.066	86.58
	6.5	3.05	0.93	.072	87.76
	4.0	3.41	0.85	.082	88.97
Golden Cushaw (cream)	9.7	5.90	0.45	.245	86.03
	9.7	5.29	0.75	.257	84.92
	17.1	7.45	0.40	.244	82.87
Averages:					
Cocozella Vegetable Marrow	4.2	0.16	6.07	.173	95.75
Zucchini Grey	4.7	0.36	12.25	.....	95.32
Early White Bush Scallop	16.7	0.06	0.55	.049	93.77
Early Summer Prolific Straightneck	12.1	.....	0.42	.....	94.25
Early Summer Crookneck	11.2	0.33	1.28	.119	91.76
Butternut	6.8	3.31	0.82	.073	87.77
Golden Cushaw	12.2	6.21	0.53	.249	84.61

Cocozella Vegetable Marrow. The ascorbic acid content of the raw Cocozella ranged from 2.1 to 7.4 mg. with an average of 4.2 mg. per 100 gm. The carotene varied from 0.10 to 0.22 mg. with an average of 0.16 mg. per 100 gm. The chlorophyll ranged from 4.10 to 7.10 with an average of 6.07 mg. per 100 gm. Thus, even though the immature Cocozella had a very thin green skin, its chlorophyll content was high. The Cocozella contained from 0.161 to 0.184 mg. with an average of 0.173 mg. of riboflavin per 100 gm. The amount of water present ranged from 95.36 to 96.05 per cent.

Zucchini Grey. The ascorbic acid values for the raw squash were 4.5 to 4.8 mg. with an average of 4.7 mg. per 100 gm. The carotene content varied from 0.33 to 0.39 mg. with an average of 0.36 mg. per 100 gm. The Zucchini was exceedingly rich in chlorophyll; values of 11.70 to 12.80 mg.

with an average of 12.25 mg. per 100 gm. were obtained. It contained 95.32 per cent of water.

Early White Bush Scallop contained a very large amount of ascorbic acid; the values were 11.0 to 25.5 mg. with an average of 16.7 mg. per 100 gm. Since the entire squash was practically colorless, a very low carotene content was anticipated and essentially identical results, 0.06 mg. per 100 gm., were obtained for the different raw squashes. The chlorophyll content was also relatively low and averaged 0.55 mg. per 100 gm. The riboflavin content was least for this variety of squash, namely, 0.049 mg. per 100 gm. The water content averaged 93.77 per cent.

Early Summer Prolific Straightneck contained 94.25 per cent of water and was rich in ascorbic acid which ranged from 10.1 to 14.4 mg. with an average of 12.1 mg. per 100 gm. Only one carotene assay, 0.40 mg. per 100 gm., was completed. The chlorophyll content of this variety of squash was lower than that of any other, and varied from 0.38 to 0.50 mg. with an average of 0.42 mg. per 100 gm.

Early Summer Crookneck was rich in ascorbic acid, 9.9 to 12.3 mg., with an average of 11.2 mg. per 100 gm. The carotene content varied from 0.20 to 0.40 mg. with an average of 0.33 mg. per 100 gm. Values of 1.10 to 1.50 mg. with an average of 1.28 mg. per 100 gm. were obtained for the chlorophyll content. Crookneck was also found to be a good source of riboflavin; different squashes contained from 0.115 to 0.125 mg. with an average of 0.119 mg. riboflavin per 100 gm. Its water content, 91.76 per cent, was somewhat lower than that of the four varieties previously discussed.

Butternut. The ascorbic acid varied from 4.0 to 10.0 mg. with an average of 6.8 mg. per 100 gm. It contained a large amount of carotene, from 3.05 to 3.46 mg., with an average of 3.31 mg. per 100 gm. The chlorophyll averaged 0.82 mg. with a range of from 0.68 to 0.93 mg. per 100 gm. Riboflavin assays gave values of 0.066 to 0.082 mg. with an average of 0.073 mg. per 100 gm. The water content, 87.77 per cent, was significantly lower than that of the other five varieties of squashes which have been considered above.

Golden Cushaw contained from 9.7 to 17.1 mg. with an average of 12.2 mg. of ascorbic acid per 100 gm. The carotene content was unusually high, varying from 5.29 to 7.45 mg., with an average of 6.21 mg. per 100 gm. It contained a relatively small amount of chlorophyll, 0.40 to 0.75 mg., with an average of 0.53 mg. per 100 gm. The riboflavin content of this variety of squashes was exceedingly high, 0.244 to 0.257 mg., with an average of 0.249 mg. per 100 gm. The water content was 84.61 per cent.

DISCUSSION

The average ascorbic acid content of the seven varieties of raw squashes varied from 4.2 mg. per 100 gm. for Cocozella Vegetable Marrow to 16.7 mg. for Early White Bush Scallop. Three values, namely, 11.2 mg. for Early Summer Crookneck, 12.1 mg. for Early Summer Prolific Straightneck, and 12.2 mg. for Golden Cushaw, are over one-half, and 16.7 mg. for Early White Bush Scallop was over four-fifths, of the value reported by Holmes, Jones, and Wertz (1944) and Holmes, Jones, Wertz, Esselen, and

McKey (1944) for milk produced in the same area. Since tomatoes are commonly cited as an excellent source of ascorbic acid, these results may be compared with those previously reported for tomatoes. In a study of varietal differences in ascorbic acid content of tomatoes Tripp, Satterfield, and Holmes (1937) found from 14.8 mg. per 100 gm. for John Baer to 21.8 mg. for Pritchard with an average of 17.7 mg. per 100 gm. for 10 varieties of vine-ripened tomatoes. Later Holmes, Jones, and Ritchie (1943) reported that late-winter tomatoes purchased in several independent and chain grocery stores contained only 8.8 mg. of ascorbic acid per 100 gm. Hence, Early Summer Crookneck, Early Summer Prolific Straightneck, and Golden Cushaw contain about 70 per cent and Early White Bush Scallop approximately 95 per cent as much ascorbic acid as vine-ripened summer tomatoes and about 140 to 200 per cent, respectively, as much ascorbic acid as later-winter tomatoes. Woodruff and Scoular (1942) found 22 to 30 mg. of ascorbic acid in Yellow Crookneck and 13 to 29 mg. per 100 gm. in Early White Bush Scallop. Slepykh (1940) found the amount of ascorbic acid decreases with the age of the squash, that the pulp content is 8.6 to 13.8 mg. per cent, the core 16.3 to 31.0 mg. per cent, and the whole squash 13.8 to 27.6 mg. per cent. Burrell and Ebright (1940) reported that White Bush and Zucchini squashes, purchased in the market, contained 0.232 and 0.237 mg. of ascorbic acid per gram.

The carotene content of the raw squashes varied widely. Early White Bush Scallop contained 0.06 mg. per 100 gm. and Golden Cushaw more than 100 times as much, 6.21 mg. per 100 gm. In general, the carotene content increased with the increase in the intensity of the yellow color of the squashes, and few vegetables contain more carotene than the Golden Cushaw squashes included in this study. The literature reviewed contained little information concerning the carotene content of summer squashes. Smith (1936) found five Sherman units of vitamin A per gram in cooked Zucchini and three Sherman units in cooked summer squash. Booher and Marsh (1941) determined the vitamin A value of fresh Summer Crookneck squash by the rat-growth method and found 700 I. U. per 100 gm. Flanagan and Salmon (1944) report that the fresh Yellow Crookneck squash grown in Alabama contains 0.84 mg. of carotene per 100 gm.

The chlorophyll content of the raw summer squashes varied from 0.42 mg. per 100 gm. for Early Summer Prolific Straightneck to 12.25 mg. for Zucchini Grey. Except for the two varieties with green skins, Cocozella Vegetable Marrow 6.07 mg. and Zucchini Grey 12.25 mg. per 100 gm., the chlorophyll content was below 1.3 mg. per 100 gm.

The riboflavin content of raw summer squashes is of particular interest, since riboflavin is considered an essential constituent of the human dietary, and it is found in large quantities in only a very few of the foods commonly eaten. The Early White Bush Scallop contributed about 33 per cent as much, the Butternut about 49 per cent as much, Early Summer Crookneck about 79 per cent as much, the Cocozella Vegetable Marrow 115 per cent as much, and the Golden Cushaw about 166 per cent as much riboflavin as reported by Holmes and Holmes (1943) for milk produced in this locality. Hodson (1940) found 0.52 microgram per gram of ribo-

flavin in summer squash purchased in local market. The analysis was made on a water-free sample dried in a hot-air tunnel. Sheets and co-workers (1944) report that fresh Patty Pan White Squash (Early White Bush Scallop) contains 0.056 mg. of riboflavin per 100 gm. which is essentially identical with 0.049 mg. per 100 gm. obtained in this study.

SUMMARY

Seven varieties of summer squashes grown in Victory gardens under conditions assumed to be typical of this locality for climatic, fertilizer, rainfall, sunshine, temperature, and cultural conditions were analyzed for their ascorbic acid, carotene, chlorophyll, riboflavin, and water content. The ascorbic acid content of the raw squashes varied from 4.2 mg. for Cocozella to 16.7 mg. per 100 gm. for Early White Bush Scallop. Four varieties—Early Summer Crookneck, Early Summer Prolific Straightneck, Golden Cushaw, and Early White Bush Scallop—contained over half as much ascorbic acid as milk, decidedly over half as much as summer tomatoes, and from 50 to 100 per cent more than late-winter tomatoes. The carotene content varied from 0.06 mg. for Early White Bush Scallop to 6.21 mg. per 100 gm. for Golden Cushaw. Two varieties of squashes, Butternut and Golden Cushaw, were extremely rich in carotene. The chlorophyll content varied from 0.42 mg. for Early Summer Prolific Straightneck to 12.25 mg. per 100 gm. for Zucchini Grey. The two squashes with green-colored skins, Cocozella and Zucchini, contained large amounts of chlorophyll. The raw squashes were rich sources of riboflavin. The Early White Bush Scallop contained about 33 per cent, Butternut about 49 per cent, Early Summer Crookneck about 79 per cent, Cocozella more than 115 per cent, and Golden Cushaw about 166 per cent as much riboflavin as milk produced in this locality. Since the soil, fertilizer treatment, and maturity of the squashes were not controlled, the above data should not be interpreted as representing true varietal differences but as representing the nutritive value of average, raw summer squashes grown under home-garden conditions.

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COMPARATIVE TENDERNESS OF REPRESENTATIVE BEEF MUSCLES

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Many studies have been made in order to determine the factors that are responsible for the tenderness or toughness of beef. According to Lehmann (1907) the mechanical strength of a muscle is directly proportional to the amount of connective tissue present; the muscles that are most active and therefore subject to the greatest strains have the largest amounts of connective tissue and are least tender. The active calf muscle from the round of the beef carcass contained five times more connective tissue than the tenderloin (psoas major), which is a much less active muscle. Other investigators who have reported a high correlation between the connective tissue content of the muscle and tenderness include Mitchell, Hamilton, and Haines (1928) and Mackintosh, Hall, and Vail (1936).

The relationship between the characteristics of the muscle fibers and tenderness has been investigated by Moran and Smith (1929). Their data show that the diameter of the muscle fiber, the area of the primary bundles, and area of the secondary bundles increase progressively in the following muscles: fillet (psoas major), rib (longissimus dorsi), top side (biceps femoris), and inside (semimembranosus). The number of fibers for each primary bundle decreased progressively in the same order. Thus, small muscle fibers, small primary bundles, and small secondary bundles would appear to be correlated with tenderness inasmuch as the muscles are listed in order of decreasing tenderness. Brady (1937) reported that there was no significant difference in the diameter of the fibers for the following muscles: triceps brachii (shoulder), longissimus dorsi (rib and loin), and adductor (round). He found that texture is dependent upon size of bundle; the larger the bundle, the finer the texture. Fine texture was an indication of tenderness.

The physical characteristics of muscle fibers change during refrigerated storage of beef. Paul, Lowe, and McClurg (1944) have shown that muscle fibers are well defined and wavy after one day's storage, whereas the fibers are broken and disintegrated at many locations after nine days' storage under refrigeration.

The effect of fat on the tenderness of beef muscle is not clearly understood. Helser, Nelson, and Lowe (1930) found that beef from feeders was less tender than beef from similar cattle after fattening, thereby indicating that the increased fat was a factor in improving tenderness; however, it is possible that there were changes in muscle fibers and connective tissue during the fattening period which may have been responsible for the meat becoming more tender. Hankins and Ellis (1939) found no significant

correlation between the fat content and tenderness of the cooked longissimus dorsi muscle of beef, from which they concluded that variations in tenderness are caused mainly by factors other than fat.

There is little agreement among investigators as to whether cooking increases or decreases the tenderness of beef. Lehmann (1907) states that the tenderness of the loin muscle did not change significantly with periods of cooking varying from 30 minutes to three hours, whereas the flank muscle improved in tenderness. Satorius and Child (1938) reported that the semitendinosus muscle of the beef round increased in tenderness during cooking until an interior temperature of 67° C. (153° F.) was reached, then the muscle became less tender on further heating. They found that the longissimus dorsi was made more tender by heating to 58°C. (136°F.) but the tenderness of the triceps brachii and adductor muscles was not affected by heating to 58°C. Satorius and Child (1938) state that the diameter of the muscle fibers decreased with increased coagulation up to 67°C., but no difference was found between meat coagulated at 67° C. and that coagulated at 75° C. (167° F.). They conclude that shrinkage owing to coagulation of the muscle fibers is complete at 67°C.

Lack of uniformity in cooking methods, cooking temperature, cooking time, muscles used, degree of fatness of the meat, age of the meat, and method of measuring tenderness account for the variable results obtained by different investigators. In addition, the tenderness of beef may be affected differentially by various factors during the cooking process.

There has been relatively little information published on the tenderness of beef muscles and on the factors that are responsible for tenderness. It was thought that a logical approach to this problem was to study a large number of muscles from the beef carcass.

EXPERIMENTAL PROCEDURE

The beef selected consisted of three heifer carcasses which averaged 500 pounds dressed weight and graded commercially "U. S. Good." On the second day following the dressing operations the carcasses were cut into standard wholesale cuts. All muscles weighing a pound or more were dissected on the second, third, or fourth days following slaughter. During this period they were stored at 1.1°C. (34°F.) and at a relative humidity of 95 per cent.

These muscles were weighed and samples were taken from the thickest portion of each muscle for histological examination and for tenderness tests. In one carcass, in order to determine if there were important differences in tenderness within individual muscles, samples were taken from the middle and both ends of each of 25 large muscles. The samples which were used for tenderness experiments were frozen at —34.4°C. (—30°F.) on the fifth day after slaughter. After one week to three weeks' storage at —30°F. the samples were removed and thawed at 2.2°C. (36°F.) in preparation for shear tests and cooking. The defrosted meat was sampled for moisture, fat, pH, and shear tests; consequently, in the case of muscles weighing about one pound, only small portions of between 100 and 200 grams were available for cooking.

The difficulties in obtaining uniform cooking of such small units by standard cooking methods are obvious. Therefore, after preliminary experiments it was decided to cook the muscles in lard which was kept at a constant temperature of 121.1°C.(250°F.). Thermometers were inserted in the meat and each piece was removed from the lard upon reaching an internal temperature of 76.7°C.(170°F.); however, the temperature of the meat around the thermometer continued to rise one or two degrees after removal. From 12 to 20 minutes were required to cook the meat under these conditions. The length of cooking time depended not only on the weight but also on the thickness of the meat.

The Warner-Bratzler shear (1928) was used to obtain shear readings on both the raw and cooked meat. Four or more readings were made at right angles to the grain on each piece of raw and cooked meat. The temperature of the raw and cooked meat when sheared was 2.2°C.(36°F.) and 23.9 to 29.4°C.(75 to 85°F.), respectively. Uniform sampling of muscles less than one inch in thickness was not possible with the conventional one-inch sampling cylinder; consequently, all samples were taken with a cylinder one-half inch in diameter. The shear on the cooked muscles was selected as a standard of tenderness in analyzing the data. Each sample of cooked meat was evaluated organoleptically for tenderness as very tender, tender, slightly tender, medium, slightly tough, tough, or very tough. These tenderness ratings were given numerical values of 2, 4, 6, 8, 10, 12, and 14, respectively.

Samples of both raw and cooked muscles for histological preparations were taken from all of the muscles studied. The part of the muscle used for this purpose was the same as that used for shear studies and chemical analyses. Small pieces of the raw muscles were fixed in 10 per cent formalin, dehydrated in alcohol, embedded in paraffin, and sectioned. Weigert's elastic connective tissue stain was used to show the distribution of the elastic connective tissue while Mallory's triple connective tissue stain was employed principally to demonstrate the collagenous connective tissue. From the cooked muscles larger samples of muscle (approximately 1½x¾ inches) were sectioned on the freezing microtome and stained with gentian violet and Sudan III.

Muscles were compared as to the relative amounts of elastic connective tissue and collagenous tissue. The following arbitrary numerical evaluations were used in making the comparison:

Relative amount of tissue present	Elastic fibers	Collagenous fibers
None	1	1
Small	3	3
Medium	5	5
Large	7	7

By adding the numerical evaluation for the elastic fibers and the collagenous fibers, a figure was obtained for each muscle which represented the histological tenderness rating. For example, a muscle with large numbers of elastic fibers and of collagenous fibers would rate 14, whereas one with small amounts of both types of fibers would rate only 6. These

histological evaluations were compared with the shear readings and with the organoleptic evaluations. Freezing microtome sections were examined and compared with regard to the general distribution and arrangement of muscle bundles and connective tissue.

These procedures and techniques were used in studies made on 25 of the larger beef muscles from each of three "U. S. Good" carcasses; the identification and approximate location of these muscles are shown (Fig. 1).

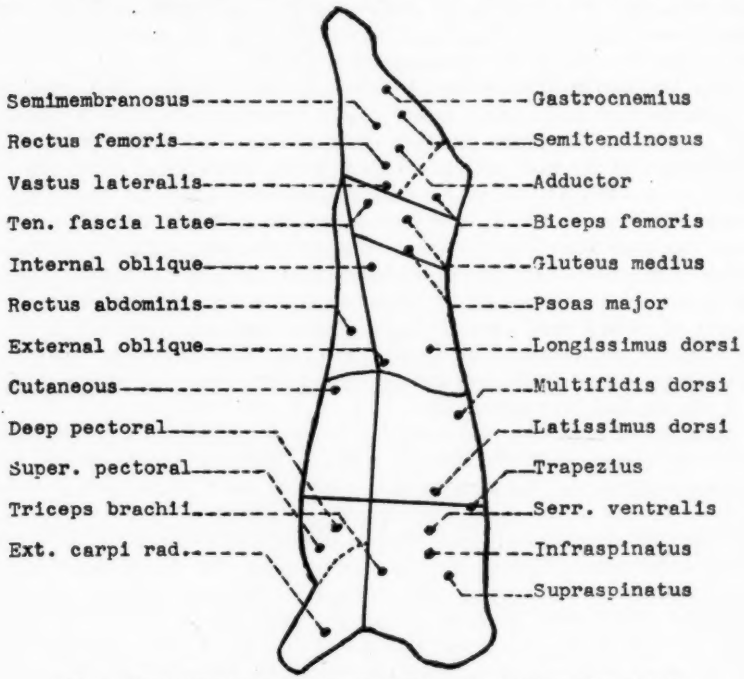

FIG. 1. Chart showing approximate location of the 25 muscles studied.

EXPERIMENTAL RESULTS

Variations in Fat, Moisture, and pH: Muscles may vary widely in fat and moisture. The external oblique muscle had an average fat content of 11.5 per cent, whereas at the other extreme, the extensor carpi radialis of the fore shank had a fat content of 1.5 per cent. As would be expected, the moisture varies inversely with the fat. The moisture in the external oblique averaged 67.4 per cent while the average moisture content in the extensor carpi radialis was 76 per cent. The pH values of muscles fell within the range pH 5.5 and 5.8, which were the figures for the semitendinosus of the round and multifidis dorsi, respectively.

Histological Variations of Muscles: Muscles varied in the amounts of collagenous and elastic tissue as well as in the amounts of fat and the size of the muscle bundles. Although in some muscles there were variations in the amounts of elastic tissue and collagenous tissue in different regions,

most of the muscles were fairly uniform from one end to the other. Collagenous and elastic connective tissue fibers were abundant in the latissimus dorsi, whereas those fibers were not extensive in the psoas major (Fig. 2). The former muscle had a high shear reading when cooked (10.7) and rated tough in organoleptic tests in contrast to the latter muscle which was very tender and had a shear reading of 7.5. Weigert's stain clearly demonstrated the abundant amount of elastic tissue in the latissimus dorsi muscle (Fig. 2) and the absence of this type of tissue in the psoas major. This variation in collagenous and elastic connective tissue no doubt has an important bearing on the problem of tenderness in beef since shear tests on predominantly collagenous tissue (thick fascia) and on predominantly elastic tissue (ligamentum nuchae) gave readings much higher than those of most muscles.

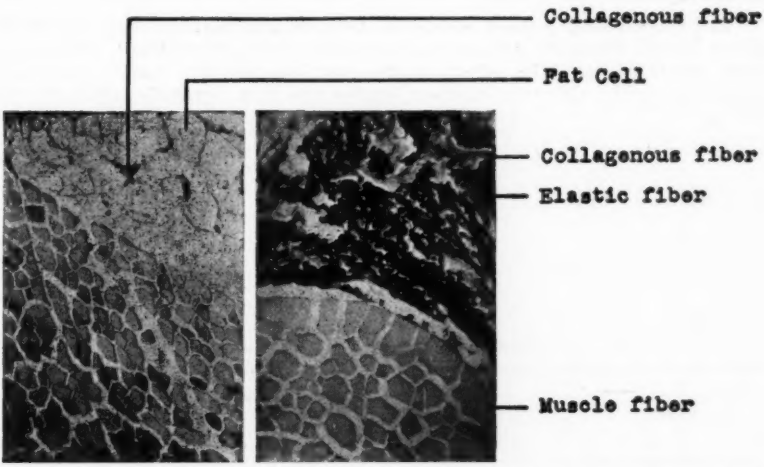

FIG. 2. Photomicrographs of the psoas major muscle (on left) and latissimus dorsi muscle (on right) showing difference in the amount of connective tissue present (135×).

There was great variation in texture of the muscles as determined by the size of the bundles of fibers (fasciculi) and the amount of connective tissue (perimysium) surrounding the bundles. In the superficial pectoral muscle (Fig. 3) there were large and well-defined fasciculi with extensive perimysia, conditions which are associated with coarse texture. In the psoas major (Fig. 3) the small amount of connective tissue was not sufficient to divide the muscle into very distinct bundles, thereby forming a muscle with smooth, fine texture. This muscle was very tender and had a low shear reading as compared with the previous muscle which was very tough and had a high shear reading. The fibers of the superficial pectoral muscle had a greater diameter than those of the psoas major. Between these two extremes of tissue arrangement were found all variations in size of bundles and amount of connective tissue. The serratus ventralis muscle (Fig. 3) of the chuck was an example of such an intermediate muscle,

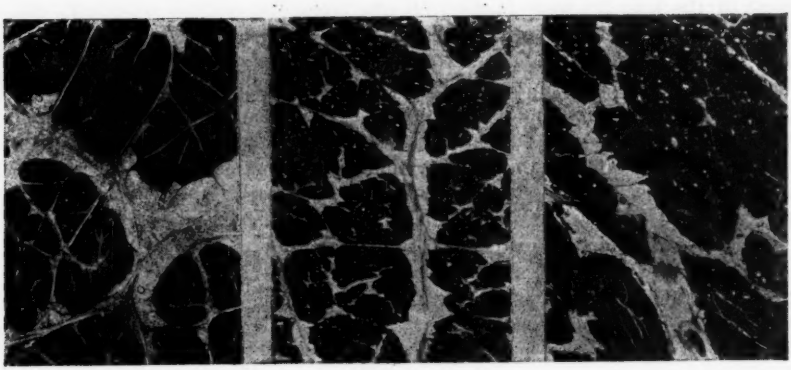

FIG. 3. Superficial pectoral muscle (on left) showing relatively large and distinct muscle bundles (fasciculi) and extensive connective tissue (perimysium). Serratus ventralis muscle (center) with smaller bundles and less connective tissue. Psoas major muscle (on right) with very indistinct bundles and with very little connective tissue (4×).

TABLE 1

Tenderness Variation Within Muscles

Muscle	Region of muscle	Average shear of cooked muscle with standard deviation (based on samples from three carcasses)
		lb.
Biceps femoris	Origin	7.5±1.1
	Middle	9.1±0.7
	Insertion	10.8±1.0
Latissimus dorsi	Origin	11.2±0.7
	Middle	12.5±1.3
	Insertion	13.0±0.6
Psoas major	Origin	7.5±0.5
	Middle	7.1±0.1
	Insertion	7.6±0.8
Deep pectoral	Origin	12.0±0.6
	Middle	12.3±1.1
	Insertion	12.0±1.0
Longissimus dorsi	Posterior end	8.3±0.8
	Middle	8.3±0.9
	Anterior end	10.7±1.4
Multifidis dorsi	Posterior end	6.9±0.3
	Middle	8.0±0.5
	Anterior end	9.2±1.2

having moderately large fasciculi and moderately thick connective tissue. This muscle rated medium in organoleptic tests and shear readings. These variations in texture agree with the results of Moran and Smith (1929) who observed small fibers and bundles in the tenderloin and large fibers and bundles in the muscles of the round. These data are also in agreement with the conclusions of Brady (1937) who associated fine texture with tenderness.

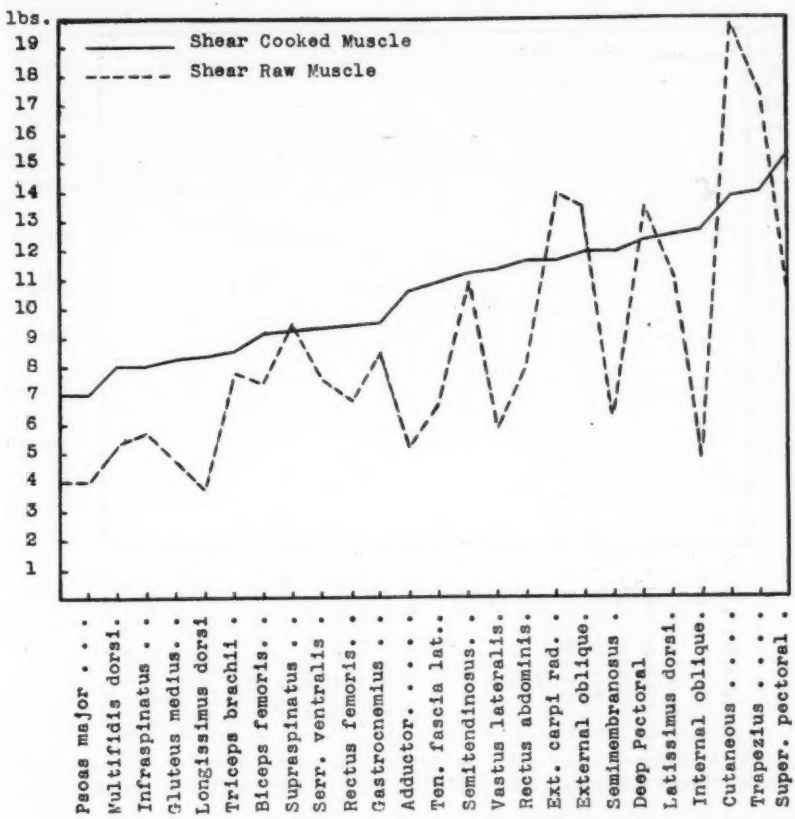

FIG. 4. Shear values of cooked muscles listed in order of decreasing tenderness with corresponding shear values for raw muscles (average of three muscles).

Observations on a number of histological preparations of cooked muscles showed a pronounced change in the collagenous connective tissue but very little change in the elastic connective tissue. In cooked meat the muscle fibers were more compact than in the raw meat and had indistinct and irregular borders, while the collagenous fibers of the cooked meat were enlarged and irregular in shape. Short periods of cooking appeared to shrink the muscle fibers and began to hydrolyze the collagenous tissue.

Tenderness Variation Within Muscles: Tenderness determinations on different parts of the same muscle occurring in different wholesale cuts indicated variations in tenderness within the muscle. Consequently, sections were cut from either end and the middle of representative muscles for the purpose of making direct comparisons on differences in tenderness. Data on representative muscles (Table 1) indicate that the biceps femoris and latissimus dorsi were found to be progressively more tender from the insertion end to the origin end of the muscle. The longissimus dorsi and

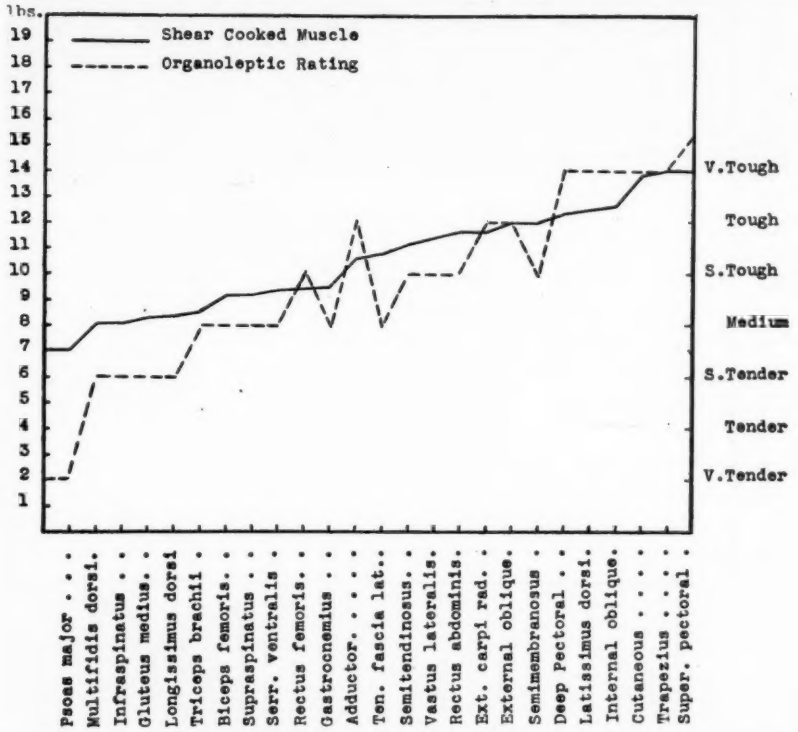

FIG. 5. Shear values of cooked muscles listed in order of decreasing tenderness with corresponding organoleptic ratings for the cooked muscles (average of three muscles).

multifidis dorsi were somewhat less tender at the anterior ends of the muscles. Most of the muscles were fairly uniform in tenderness throughout the length of the muscle. The psoas major and deep pectoral muscles were representative of such muscles which were uniformly tender and tough, respectively.

Tenderness Variation Between Muscles: In Figs. 4, 5, and 6 the shear of the raw muscles, organoleptic rating, and histological rating, respectively, have been compared with the shear of the cooked muscle. Correlation coefficients for the data presented (Figs. 4, 5, and 6) were 0.6, 0.9, and 0.7, respectively. All of these coefficients are significant, according to

Snedecor (1940), particularly the coefficient between the shear of the cooked meat and the organoleptic rating. Shear values on circular one-half inch samples of cooked meat ranged from seven pounds to over 15 pounds (Fig. 4). Shear readings on raw muscles varied from 3.8 to over 20 pounds. The shear values on the raw meat tended to increase as the shear values on the cooked meat increased, although there were many exceptions to this trend. The data (Fig. 5) agree with the results of other investigators,

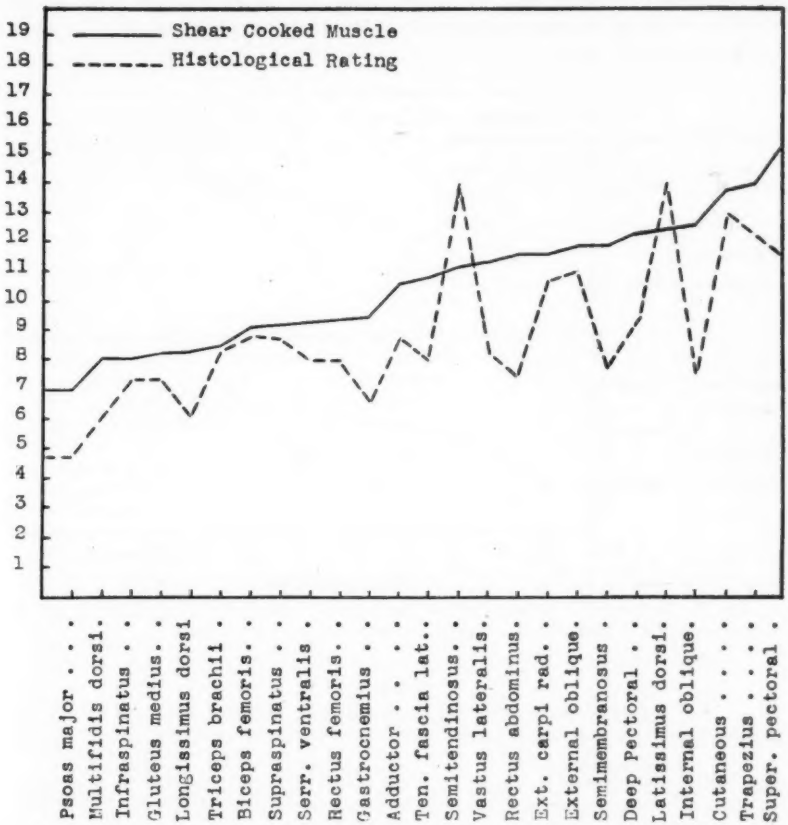

FIG. 6. Shear values of cooked muscles listed in order of decreasing tenderness with corresponding histological ratings for raw muscles (average of three muscles).

Mackintosh, Hall, and Vail (1936), showing that there was a high correlation between the shear on the cooked meat and the organoleptic rating. The shear readings on cooked tender muscles appeared to be higher than the organoleptic rating indicated, if the latter is accepted as the final measure of tenderness (Fig. 5).

For the most part, muscles with small amounts of connective tissue had low shear readings, while muscles with large amounts of connective tissue had higher shear readings (Fig. 6). The relative amounts of col-

lagenous and elastic tissue were plotted against the shear readings and a definite correlation was observed (Fig. 7). A higher correlation was indicated between the amounts of collagenous and elastic fibers and the raw shear reading than between the amounts of collagenous and elastic fibers and the cooked shear readings. Although abundant connective tissue tended to increase the shear readings of muscles, there appear to be other important factors affecting tenderness, since the histological ratings of certain muscles are widely divergent from the shear values. The change in shear values during cooking is difficult to explain on the basis of connective tissue alone.

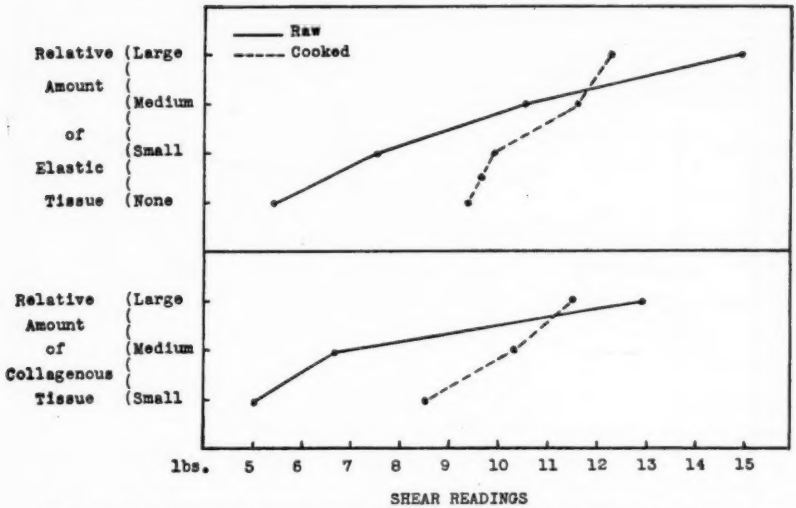

FIG. 7. Relationship between shear readings on raw and cooked muscles and the relative amounts of elastic and collagenous tissue in the raw muscle.

TABLE 2

Effect of Amount of Intramuscular Fat on Shear Readings

Amount of fat in muscle sample	Fat—average of 8 samples	Average shear readings with standard deviation	
		Raw muscle	Cooked muscle
	pct.	*lb.*	*lb.*
Large amount	8.3±2.7	8.3±2.6	8.6±1.2
Medium amount	6.5±2.0	8.0±2.9	7.9±2.0
Small amount	4.5±1.0	8.9±3.9	9.7±2.1

Effect of Intramuscular Fat on Shear Readings: In order to determine the effect of variations in the amount of intramuscular fat on tenderness, three sections were cut from different locations in each of eight muscles. Part of each section of the muscle was used for fat determination and the remainder of the section was tested for tenderness in both the raw and cooked states. The data (Table 2) show that there was

no relationship between the amount of fat within the muscle and the shear of the raw or cooked muscle.

Fat as it occurs in kidney suet offers no resistance to the Warner-Bratzler shear, while the fat which covers the brisket is tougher than most muscles (Table 4). The protein content of kidney fat and brisket fat was 0.69 and 3.81 per cent, respectively, which indicated that brisket fat contained more connective tissue. Differences in the amount of connective tissue associated with the intramuscular fat may explain why it was not possible to show a positive relationship between high fat content of muscles and low shear values.

TABLE 3

Effect of Heat on Tenderness (Shear) of Muscles

Muscle	Shear readings—average of 3 muscles		Difference	Change
	Raw muscle	Cooked muscle		
	lb.	*lb.*	*lb.*	*pct.*
Internal oblique	4.7	12.6	7.9	168.1
Longissimus dorsi	3.8	8.3	4.5	118.4
Adductor	5.2	10.6	5.4	103.8
Vastus lateralis	5.8	11.3	5.5	94.8
Semimembranosus	6.3	11.9	5.6	88.8
Psoas major	4.0	7.1	3.1	77.5
Gluteus medius	4.7	8.2	3.5	74.5
Tensor fascia latae	6.7	10.8	4.1	61.2
Multifidis dorsi	5.3	8.0	2.7	50.9
Superficial pectoral	10.4	15.3	4.9	47.1
Rectus abdominis	8.1	11.6	3.5	43.2
Infraspinatus	5.7	8.0	2.3	40.4
Rectus femoris	6.8	9.4	2.6	38.2
Serratus ventralis	7.5	9.3	1.8	24.0
Biceps femoris	7.4	9.1	1.7	23.0
Gastrocnemius	8.4	9.5	1.1	13.1
Latissimus dorsi	11.2	12.5	1.3	11.6
Triceps brachii	7.8	8.5	0.7	8.9
Semitendinosus	10.9	11.1	0.2	1.8
Deep pectoral	12.5	12.3	—0.2	—1.6
Supraspinatus	9.5	9.2	—0.3	—3.1
External oblique	13.4	11.9	—1.5	—11.2
Extensor carpi radialis	13.9	11.6	—2.3	—16.5
Trapezius	17.4	14.0	—3.4	—19.5
Cutaneous	26.0	13.8	—12.2	—46.9

Effect of Heat on Tenderness of Muscles: Twenty-five muscles are listed according to the percentage change in shear readings brought about by cooking (Table 3). Most of the muscles were made less tender by cooking, while some did not change significantly and others became more tender. The internal oblique muscle had a shear reading of 4.7 pounds on the raw meat and a shear reading of 12.6 pounds on the cooked meat, or on the basis of the shear readings the muscle was made 168 per cent tougher by cooking. The shear readings on the deep pectoral were 12.5 pounds on the raw meat and 12.3 pounds on the cooked meat. Therefore the shear readings indicated that this muscle was similar in tenderness

in both the raw and cooked states. At the other end of the list, the cutaneous muscle had a shear reading of 26 pounds on the raw meat and a shear reading of 13.8 pounds on the cooked meat. This would indicate that heating quickly to 76.7°C.(170°F.) improved the tenderness of the cutaneous muscle.

In the present experiments, the meat was cooked in lard at a temperature of 121.1°C.(250°F.). Consequently, an inside temperature of 170°F. was reached in a much shorter time than if the meat had been cooked in a 162.8°C.(325°F.) oven. This was demonstrated in preliminary experiments which indicated that the oven-cooked meat was consistently more tender and there was less variation in tenderness between muscles.

Relative Tenderness of Connective Tissue, Fatty Tissue, and Muscle: The relative tenderness of connective tissue, fatty tissue, and muscle is compared (Table 4). Samples of white connective tissue one-half inch in

TABLE 4

Relative Tenderness of Connective Tissue, Fatty Tissue, and Muscle

Identification	Average of 12 shear readings with standard deviation	
	Raw sample	Cooked sample
	lb.	*lb.*
Collagenous connective tissue (infraspinatus and biceps femoris)	120±.....	21.5±3.4
Elastic connective tissue (ligamentum nuchae)	81.1±5.0	42.3±4.7
Cover fat with large amounts of connective tissue (brisket)	25.2±4.5	4.7±0.4
Cover fat with small amounts of connective tissue (sirloin)	12.4±3.6	3.0±1.3
Kidney fat	0.0±.....	0.0±.....
Representative muscle (rectus femoris)	6.8±2.5	9.4±1.5

diameter from the infraspinatus and biceps femoris were so tough that more force was required to shear them than the maximum capacity of the shear, which was 120 pounds. On cooking, white connective tissue improved in tenderness so that only 21.5 pounds were required to shear a sample. Yellow connective tissue (ligamentum nuchae), which contains more elastin and less collagen than white connective tissue according to Mitchell, Zimmerman, and Hamilton (1927), had a shear reading of 81.1 pounds, but when cooked it still required a force of 42.3 pounds to shear the sample. Therefore, the yellow connective tissue improved relatively less in tenderness than did the white connective tissue. Fatty tissue, irrespective of the amount of connective tissue, improved materially in tenderness on cooking. On the other hand, the rectus femoris, a representative muscle, became tougher on cooking. Therefore, when meat is cooked quickly to 170°F. it becomes obvious that factors other than connective tissue and fat have a profound effect on tenderness. These factors include coagulation and denaturation of proteins together with varying degrees of hardening and shrinkage of the fibers. Grouped together, these

changes appear to exert a greater negative effect on tenderness than the positive effect of partial collagen hydrolysis.

SUMMARY

Studies were made on the comparative tenderness of 25 representative muscles from three "U. S. Good" beef carcasses. Muscle tenderness was measured by shear readings on the raw and cooked meat, by organoleptic ratings, and by histological ratings based upon the relative amounts of collagenous and elastic tissue in the muscle. The muscles were listed according to tenderness.

Shear readings on the cooked meat were positively correlated with shear readings on the raw meat, organoleptic ratings, and histological ratings.

It was found that tenderness varied from muscle to muscle and in a few instances there was variation in tenderness within the muscle.

Most of the 25 muscles used in this study decreased in tenderness on cooking. Inasmuch as connective tissue and fatty tissue are made more tender by cooking, the decrease in tenderness of the muscle may be associated with factors such as coagulation and denaturation of muscle proteins together with varying degrees of shrinkage and hardening of the muscle fibers.

ACKNOWLEDGMENT

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STUDY OF QUALITY IN TEMPLE ORANGES

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During the last 10 years the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, has conducted investigations at Orlando, Florida, on seasonal changes in oranges, Harding, Winston, and Fisher (1940), grapefruit, Harding and Fisher (1945), tangerines, and Temple oranges. These studies included changes in the physical and chemical constituents that occurred in the principal varieties of the fruits as they matured and ripened on the tree, and the factors that influenced eating quality and food value.

The purpose of the present investigation is (1) to study the palatability of Temple oranges, its seasonal trend and connection with factors more exactly measurable, and (2) to study the method used in evaluating palatability, and possibilities in statistical treatment of ratings.

The subject of tasting tests is being given widespread attention at present in food research. The extent to which qualitative ratings can be standardized, given a quantitative interpretation, and analyzed statistically is of great interest. Recent discussions include one by Howe and Barbella (1937) on meat flavor and one by Levin (1943) on dried-egg quality. The latter is similar in viewpoint to the study here reported. Fisher (1941) outlines a method believed to be of great promise in dealing with personal and qualitative ratings, but it is beyond the scope of the present study. In efforts to make such ratings more quantitative it has been found of value to limit the number of grades, to define and describe them carefully, and to check them occasionally against a standard where this is possible. These measures tend to standardize conditions for readings for all workers and occasions.

EXPERIMENTAL PROCEDURE

The Temple oranges for this investigation were obtained from four experimental plots located in commercial groves at Windermere, Florida, where the soil type is Norfolk fine sand. Each plot consisted of 15 to 25 trees, and the trees of each plot were on a different rootstock, namely, rough lemon, sour orange, sweet orange, or Cleopatra (spicy tangerine). The trees were of mature age. Tests on the fruit were made at seven different picking periods and thus included a wide range of stages in fruit development and ripening, from immaturity to senility. Picking was in the first five days of each month, October to April.

It will be noted that only one plot was available for each rootstock, hence the experiment is subject to the criticism that plot replication is not provided; that we are not sure whether differences found are characteristic of rootstocks or of the plots themselves. However, the records may be used in preliminary study. The differences found in this work between fruit from different rootstocks are such as have been repeatedly noted before.

The fruit samples were taken to the laboratory at Orlando, Florida, immediately after they were picked and were placed in storage at 0°C. (32°F.) until tested. Each sample consisted of 60 or more fruits picked from 15 to 25 trees. The total-solids content and total-acid content were determined on the composited juice of 25 fruits. The fruits that remained were used for palatability tests.

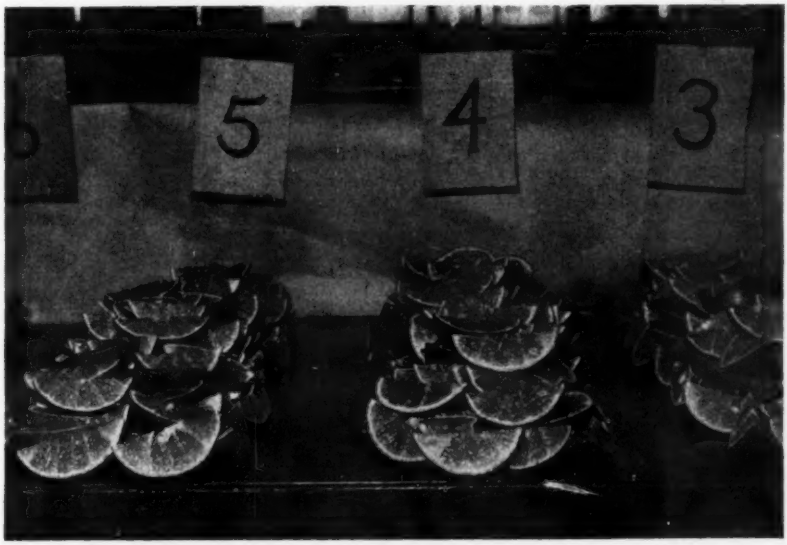

FIG. 1. Samples of citrus fruit arranged on laboratory table for taste evaluation. Each judge was advised to taste several pieces before rating a sample according to the arbitrary scale shown in the rating form (Fig. 2).

Total water-soluble solids were determined with an Abbe (Bausch and Lomb) refractometer. Total acid was determined by titration of the orange juice with standard NaOH, using phenolphthalein as an indicator, the results being calculated as anhydrous citric acid. Palatability was determined by the method described later in this paper.

FACTORS INFLUENCING QUALITY AND METHOD OF EVALUATING PALATABILITY

The internal quality of citrus fruit is influenced by several factors, such as total solids, total acid, ratio of solids to acid, texture of flesh, and aromatic constituents. The age of the fruit is also important. Immature

fruit is usually very acid or tart and has a raw, immature taste, whereas overripe fruit held on the tree too long may become insipid or develop disagreeable off-flavors.

Throughout this investigation assays for flavor or taste were made on the fruit soon after picking. In each test 30 to 50 Temple oranges were used. The fruits were cut transversely and from each half was cut a wedge-shaped piece for tasting (Fig. 1). Each judge was advised to taste several pieces before rating a sample according to the arbitrary scale shown in the rating form (Fig. 2).

Arbitrary standard	Taste or flavor of fruit	Numerical rating range corresponding to description	Individual numerical rating
Very acid	Very acid, raw, immature flavor	20-39	
Acid	Acid, with absence of raw, immature flavor	40-59	
Tart	Too tart for consumer approval	60-69	
Pleasantly tart	Minimum stage of acceptability for consumer	70-79	
Pleasantly tart to sweet	Pleasant blend of sugars and acid, with very good texture and flavor	80-100	
Insipid (aged)	Very sweet, watery, lacking in flavor, low acidity, aged	50-100	

FIG. 2. Rating form given to taste judges, who were informed that fruit rated 70 or below was regarded as not meeting consumer approval (see Fig. 1).

Staff members of the Bureau of Plant Industry, Soils, and Agricultural Engineering and of the Bureau of Entomology and Plant Quarantine stationed at Orlando regularly officiated as taste judges. Frequently visitors also were present, and they too were invited to score the various samples.

Thus judges available at each time were utilized, and the group was not the same every time. However, there were eight tasters who participated in every test. In all, 34 different men participated in one or more of the tests; 21 to 27 worked each time. Scoring was according to the scale described (Fig. 2).

At the beginning of the season the arbitrary standard scale to be used and the method of evaluating the internal quality of Temple oranges were discussed with a number of the staff members who were to participate as taste judges. At that time it was decided the numerical value of 70 would be the minimum standard of acceptability and that any fruit rated below that value would be considered undesirable. On the other hand, desirable internal quality was rated from 70 to 100 as indicated. The scale permitted the scoring of insipid fruit (values 50 to 100), but it was understood that any of the samples of insipid fruit that were rated below 70 would not meet consumer approval.

There were some variations among individual fruits in the samples, but generally any given sample was rather uniform in flavor. However, in scoring, each judge was advised to base the rating on the average flavor, after tasting several pieces of fruit of the lot.

ANALYSIS OF RATINGS

The tasters were believed to represent fairly well the prospective consumers of Temple oranges. The percentage grades given are subject to the criticisms of all such ratings; they may be somewhat subjective, difficult to standardize, and hard to keep constant from judge to judge and from time to time. There may be some doubt that they fully meet the assumptions required for analysis of variance. However, every effort was made to make the ratings sound measures of acceptability, and they may well be analyzed for what they can show.

TABLE 1

Average Grades Given Temple Oranges by All Tasters

Rootstock	Period of picking, during first 5 days of							Mean
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	
Rough lemon	27	55	78	91	95	94	83	75
Sour orange	30	58	79	93	98	96	91	78
Sweet orange	28	57	80	94	98	96	92	78
Cleopatra	27	56	81	95	98	96	92	78
Mean	28	56	79	93	97	95	89	77

The differences between tasters proved not to be large. Hence, the average grades for each month and each rootstock are presented even though the group of tasters was not exactly the same for any two months (Table 1).

TABLE 2

Grades Given Temple Oranges by Eight Tasters in Seven Periods, Averaged for Four Rootstocks

Taster	Period of picking, during first 5 days of							Mean
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	
A	28	66	74	96	95	98	93	79
B	21	38	84	96	97	98	96	76
C	24	55	59	82	99	98	94	73
D	29	58	72	95	99	98	82	76
E	20	28	87	97	96	92	60	69
F	23	64	68	99	98	96	93	77
G	38	65	91	100	98	98	92	83
H	24	42	79	100	100	98	88	76
Mean	26	52	77	96	98	97	87	76

Next a study was made of the ratings by the eight judges who worked in every period. The average rating for all four rootstocks by each of these tasters in each month is presented (Table 2); the detailed ratings are shown in Table 8, presented at the end of the paper.

The results from these eight, when averaged, were very close to those of the larger group shown (Table 1). These results gave a balanced table making possible the use of detailed analysis of variance.

While rootstock and period are reproducible sources of variation, tasters are an uncontrolled source of variation involving sampling. The interaction of rootstock and taster is logically the error for rootstocks; the interaction of taster and period, for periods. The "F" test is appropriate

for these comparisons. It will be noted that duplicate ratings were not made; if they had been, an additional line could have appeared in Table 3, that of variance for ratings within subclasses. This variance could have been regarded as the error for tasters and interactions involving tasters. It would not be expected to be higher than the rather low variance for rootstock x tasters, and period x rootstock. In absence of an estimate of this variance some inferences must be made.

TABLE 3

Summary of Analysis of Variance of Grades Given Temple Oranges by the Eight Tasters Participating in All Tests

Source of variation	Degrees of freedom	Mean square
Between periods	6	24098[1]
Between tasters	7	480[1]
Between rootstocks	3	140[2]
Interactions:		
Period x rootstock	18	31[2]
Period x taster	42	268[1]
Rootstock x taster	21	28[2]
Period x rootstock x taster	126	15

[1] Highly significant. [2] Significant.

Periods show a very marked difference of undoubted reality, mostly associated with a seasonal trend. Tasters show a difference which is undoubtedly real (statistically significant) but not very pronounced. This difference is compared with triple interaction in absence of variance between duplicates. It is practically all due to rather consistent high ratings by Taster G and low ones by E (Table 2). Differences between rootstocks, averaged through the season, are significant when compared with their appropriate error, rootstock x taster. While moderate, these differences appear significant because of the consistency with which tasters placed the rootstocks in the seasonal averages. The differences are practically all due to inferiority in rough lemon.

While, in seasonal averages, tasters were rather consistent in ranking rootstocks, the interaction of taster and period is rather high compared with triple interaction. This shows evidence of shifts of tasters in relative ratings during the season, but not enough to change ranking of tasters.

Some of these considerations show the difficulty noted in making ratings objective and comparable and in applying analysis of variance. It will also be noted that variation is greater with low or medium than with high quality (Table 2).

LABORATORY DETERMINATIONS

Acidity and solids were determined as described and are presented to show their general trends.

Analysis of variance was used in study of the acid and solids determinations, separating variance for rootstocks, periods, and interaction. There was only one determination for each rootstock-period unit, and no true error term. However, interaction variance may be tentatively used as the error estimate; it would not be expected to be lower than error.

TABLE 4

Total-Acid (as Anhydrous Citric Acid) Content of Temple Oranges, Windermere, Florida, 1943-44

Rootstock	Picking periods						
	Oct. 1-5	Nov. 1-5	Dec. 1-5	Jan. 1-5	Feb. 1-5	Mar. 1-5	Apr. 1-5
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Rough lemon	2.65	1.80	1.31	1.01	0.85	0.77	0.63
Sour orange	2.68	1.88	1.45	1.07	1.02	0.83	0.77
Sweet orange	2.49	1.79	1.36	1.09	1.00	0.93	0.76
Cleopatra	2.63	1.75	1.35	1.06	0.93	0.82	0.71

TABLE 5

Total-Solids Content of Temple Oranges, Windermere, Florida, 1943-44

Rootstock	Picking periods						
	Oct. 1-5	Nov. 1-5	Dec. 1-5	Jan. 1-5	Feb. 1-5	Mar. 1-5	Apr. 1-5
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Rough lemon	7.57	8.70	9.37	10.05	11.38	11.87	12.12
Sour orange	8.72	9.40	11.17	11.70	11.98	12.71	13.32
Sweet orange	7.98	8.90	11.57	11.65	12.23	13.36	13.42
Cleopatra	7.98	9.25	10.87	12.04	12.58	13.41	14.07

TABLE 6

Analysis of Variance of Acidity and Solids

Source of variation	Degrees of freedom	Mean square	
		Acidity	Total solids
Rootstocks	3	0.012[1]	2.55[2]
Picking periods	6	1.805[2]	14.67[2]
Interaction:			
Period x rootstock	18	0.003	0.16

[1] Significant. [2] Highly significant.

TABLE 7

Correlation Coefficients of Average Palatability Ratings With Acidity and Solids

Source of variation	Degrees of freedom	Correlation coefficient of palatability	
		Acidity	Total solids
Total	26	—0.95[1]	0.87[1]
Rootstocks	2	0.74	0.97[2]
Periods	5	—0.96[1]	0.91[1]
Interaction:			
Period x rootstock	17	0.17	0.01

[1] Highly significant. [2] Significant.

It will be seen that rootstocks showed only very moderate differences in solids and acidity, though with the consistency shown they appear significant. Both solids and acidity differed markedly from month to month.

TABLE 8

Grades Given Temple Oranges by Eight Tasters in Seven Periods for Four Rootstocks

Rootstock	Taster	Period of picking, first 5 days of						
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Rough lemon	A	25	69	73	90	90	95	82
	B	20	38	81	96	96	97	94
	C	25	40	70	85	100	95	90
	D	25	55	70	85	95	95	70
	E	20	23	85	90	95	90	60
	F	25	60	67	98	99	95	85
	G	38	65	90	98	95	95	90
	H	20	35	85	100	100	98	80
Sour orange	A	24	65	72	95	97	98	95
	B	21	39	83	95	97	100	96
	C	20	70	45	85	100	100	95
	D	25	60	75	100	100	100	85
	E	22	30	83	100	95	90	60
	F	20	69	70	99	99	96	99
	G	40	65	90	100	100	95	93
	H	35	60	80	100	100	98	95
Sweet orange	A	22	68	74	98	97	99	96
	B	22	38	85	97	95	97	96
	C	25	55	55	75	95	95	90
	D	35	55	70	95	100	100	85
	E	20	30	90	98	95	95	60
	F	21	65	68	99	95	98	95
	G	40	65	95	100	100	100	93
	H	20	40	75	100	100	100	96
Cleopatra	A	39	60	75	100	96	100	98
	B	20	37	86	98	99	98	98
	C	25	55	65	85	100	100	100
	D	30	60	75	100	100	95	90
	E	20	30	90	100	97	95	60
	F	25	64	69	99	100	97	93
	G	35	65	90	100	97	100	90
	H	20	35	75	100	100	98	80

CORRELATION WITH PALATABILITY

Both acid and solids readings were correlated with the palatability grades, using for the latter the average of all the eight tasters participating in every test. Since the set of 28 readings was classified by periods and rootstocks, the correlations were determined from covariance analysis, showing the relations in the different classifications (Table 7).

It will be observed that correlation of both solids and acid with palatability is almost altogether due to the month-to-month changes. The linear correlation with solids is lower than that with acids, although it seems more definite in agreeing with observed tendencies of rootstock differences. The correlation of solids and palatability, between rootstocks,

reaches significance without much to spare. The acidity correlation between rootstocks was in the reverse direction from that between periods and did not reach significance. No correlation was found in the interaction classification; nearly all variation is associated with period and rootstock.

On plotting monthly means of palatability ratings against acidity and against solids, it was seen that the former trend is fairly linear, while the latter shows a more definite curvilinearity. Because of this a parabola was fitted to the solids-palatability relation using the seven monthly means of each variable. This gave a correlation index of 0.984, with four degrees of freedom. The tendency to curvature (owing to down-turn late in the season) is found to be significant. The regression equation for monthly mean values was as follows: palatability grade $= -482.0 + 93.0\ S - 3.75\ S^2$, where S is percentage of solids.

Palatability ratings thus show a close negative association with acidity in the month-to-month change but not between rootstocks. They show a close positive association with total solids, both as between months and between rootstocks. In the month-to-month correlation the relation is apparently a curved one, a peak of palatability coming between 12 and 13 per cent solids. The computed maximum of the curve is at 12.4 per cent solids.

CONCLUSIONS

Palatability ratings of Temple oranges show a marked seasonal trend, palatability rising rapidly through the late fall and early winter, reaching a peak in late winter, and dropping off a little in early spring. It is concluded that differences between rootstocks were not marked, but there was indication of some inferiority in rough lemon. Tasters showed differences but not large ones. Acidity and solids showed marked seasonal trends, the former decreasing, the latter increasing; and rootstocks gave only moderate differences in acidity or total solids of the fruit. Palatability was highly correlated with both acidity and solids. The relation between palatability and content of solids seemed more consistent than the palatability-acidity relation, and could be represented by a curved graph, palatability increasing with solids up to about 12½ per cent and falling off with further increase of solids. Some features of analysis indicate difficulties in securing objectivity in the ratings. The definite association between palatability ratings and some more exact measurements indicates, however, that the ratings are rather satisfactory in measuring quality.

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EFFECT OF STEAM AND HOT-WATER BLANCHING ON ASCORBIC ACID CONTENT OF SNAP BEANS AND CAULIFLOWER[1]

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The fact that vegetables which are to be preserved by quick freezing and freezer-locker storage should be subjected to a preliminary blanching or scalding process is generally recognized. This study was undertaken in order to compare the effects of hot-water and steam blanching as practiced in the home on the ascorbic acid content of snap beans and cauliflower. Determinations of ascorbic acid content were made during the preparation for freezing, after quick freezing, and at intervals during freezer-locker storage.

Few studies have been published comparing hot-water and steam methods of blanching. Brown (1941) stated that hot water is a more effective blanching agent than steam because it separates the particles, reaches all surfaces, and has a high thermal capacity. He added that steam should be used for blanching vegetables that have extremely large surfaces exposed to leaching action. The effect of blanching prior to canning of fresh peas, broad beans, stringless and runner beans, carrots, potatoes, parsnips, sprouts, swedes, dried peas, and dried beans has been studied by Adam and Horner (1941). They reported the average retentions of ascorbic acid in all the vegetables tested as 72, 64, and 58 per cent for the one-, three-, and six-minute water blanch, respectively, and 75 per cent for the material blanched in steam for three minutes. Melnick, Hochberg, and Oser (1944) concluded that steam blanching is to be preferred for snap beans since the hot-water method resulted in a notable loss of soluble nutrients while the loss during steam blanching was not significant. Farrell and Fellers (1942) found that 33 per cent of the total ascorbic acid content of Bountiful snap beans was lost during preparation of the beans for freezer storage.

No pertinent data have been found concerning the effect of blanching on the ascorbic acid content of cauliflower.

EXPERIMENTAL PROCEDURE

The Stringless Black Valentine snap beans used in this study were grown under the supervision of the Department of Horticulture. Series of plantings were made in both 1942 and 1943 so that the snap beans

[1] This paper results from a study which was carried on for two years, 1942 and 1943. The data obtained in 1942 represent a portion of the thesis submitted by Janet L. Retzer to the Graduate School of the University of Illinois in partial fulfillment for the degree of Master of Science.

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might be at approximately the same stage of maturity when they were processed. The beans were brought to the Food Research Laboratory immediately after picking and were prepared for freezing as rapidly as possible. They were thoroughly washed and dried with towels, the cap and stem ends were removed, and the beans cut into pieces approximately one and one-half inches in length.

Cauliflower of unknown source and variety was purchased from the local market from October 5 to November 3 in 1942 and 1943. The untrimmed heads were loosely packed in a refrigerator, held at a temperature of approximately 5°C.(41°F.) and kept there until used. In preparation, the cauliflower heads were trimmed, thoroughly washed, separated into flowerlets approximately one-half to one inch by three-fourths to one and one-half inches in size, and dried on towels. Stainless-steel knives were used for all cutting procedures.

Weighed portions of the vegetables were tied in loosely formed, single-layer, cheesecloth bags and blanched as indicated (Table 1). The samples

TABLE 1

Blanching Data for Snap Beans and Cauliflower

Vegetable	Number of bags blanched at one time	Weight of vegetable per bag	Blanching medium	Blanching time	Temperature of water at end of blanching period
		gm.		*min.*	*°C.*
Snap beans	3	240	Steam	4½	....
	5	240	8 quarts of water	3	93
Cauliflower	2	275	Steam	5½	....
	3	275	11 quarts of water	4	99

which were blanched in hot water were placed in a galvanized wire basket and immersed in an enamel kettle containing boiling water. Heating was continued during the blanching process. A closely fitted, two-piece, aluminum steamer of household size was used for steam blanching. The bottom of the top half of the steamer was of an over-all perforated design allowing free passage of steam upward and condensed moisture downward.. To obtain the needed number of samples it was necessary to repeat the blanching procedure several times each day of the study. Immediately after blanching, the vegetables were cooled for three minutes in cold, continuously running tap water. After draining for half a minute, each portion was taken from the cheesecloth bag, partially dried, and packed in a cellophane-lined, pint container. The containers were heat sealed and held in the refrigerator until the lot was completed and then the cartons were placed in a quick-freezing unit of the plate type. The temperature of the freezing unit ranged from —24 to —30°C.(—11.2 to —22°F.). After 16 to 24 hours in the freezing unit the boxes were transferred to freezer-locker storage where the average temperature was approximately —17.8°C.± 2.8° (0°F.± 5°). Each vegetable was prepared for freezing and freezer-locker storage four times by both methods.

The vegetables were sampled for ascorbic acid determinations at the following stages: (1) raw, (2) blanched, (3) blanched and cooled, (4) quick frozen (after 16 to 20 hours in the freezing unit), and (5) at four periods during freezer storage. In sampling the raw beans, three cross sections from each of six whole beans were used. Eighteen pieces of snap bean, nine cross sections from the cut ends and nine from the centers of the pieces, were taken for the treated bean samples. Six flowerlets of cauliflower were used; three longitudinal sections were taken from each flowerlet and every sixth section was an outside one. Approximate eight-gram samples of snap beans and seven-gram samples of cauliflower were used.

Ascorbic acid was determined according to the method of Van Duyne, Chase, and Simpson (1944), which is based on the titration method of Tillmans, Hirsch, and Hirsch (1932) as modified by Bessey and King (1933) and Bessey (1938). The snap-bean and raw-cauliflower samples required six extractions for complete removal of the ascorbic acid.

RESULTS AND DISCUSSION

The values for the ascorbic acid content of snap beans and cauliflower are given in milligrams per gram of edible portion as determined. The weights of the raw lots and their weights after treatment were used in calculating the values for per cent retention; hence, these figures represent the total amount of ascorbic acid retained.

The mean values and standard deviations of the means for the ascorbic acid content of fresh, raw Stringless Black Valentine snap beans used in 1942 and 1943 were 0.18 ± 0.009 and 0.20 ± 0.005 milligram per gram, respectively, while the mean values for cauliflower were 0.90 ± 0.020 and 0.81 ± 0.032 milligram per gram. These ascorbic acid values for raw snap beans are similar to the averages reported by Mack, Tapley, and King (1939) and Feener, Palmer, and Fitzgerald (1937). The ascorbic acid content of raw cauliflower as given in the literature ranges from 0.28 milligram per gram, Fitzgerald and Fellers (1938), to 1.01 milligrams per gram, Rudra (1937). The values found in this study are in agreement with those reported by Wheeler, Tressler, and King (1939).

Decreases in the amounts of ascorbic acid present in snap beans and cauliflower occurred during the processes preparatory to freezing and during freezer-locker storage after both types of blanching (Table 2). Percentage retentions were not calculated for the values obtained in 1942 because some of the samples were not weighed after treatment. In the case of snap beans, the changes were small and gradual. After the second month of freezer-locker storage significant differences were found between the ascorbic acid content of raw snap beans and that of frozen, stored snap beans. On comparing the amounts of ascorbic acid present after blanching, blanching and cooling, freezing, and during freezer storage, it is apparent that the values for the hot-water-blanched products are not significantly different from those of the steam-blanched products.

In cauliflower, a vegetable of higher ascorbic acid concentration, the differences are more pronounced. Cauliflower blanched by the steam

method contained 0.83 ± 0.023 milligram per gram which is a significantly larger amount of ascorbic acid than the 0.70 ± 0.020 milligram per gram in the cauliflower blanched in hot water. However, the steam-blanched product lost a larger amount of ascorbic acid during the cooling process so that, when ready for freezing, the water-blanched cauliflower contained 0.62 ± 0.025 milligram per gram ascorbic acid and the steam-blanched cauliflower, 0.68 ± 0.034 milligram per gram. This difference is not a significant one when analyzed statistically, neither are any of the differences between the values obtained from the stored samples prepared by the two methods of blanching.

TABLE 2

Effect of Home Methods of Hot-Water and Steam Blanching on Ascorbic Acid Content of Quick-Frozen Snap Beans and Cauliflower—1942

Treatment	Snap beans		Cauliflower	
	Ascorbic acid		Ascorbic acid	
	Number of determinations	Mean and standard deviation of the mean[1]	Number of determinations	Mean and standard deviation of the mean[1]
		mgm./gm.		*mgm./gm.*
Raw	18	.18±.009	12	.90±.020
Water blanched	18	.16±.006	12	.70±.020[2,3]
Water blanched, cooled	18	.16±.006	12	.62±.025[3]
Water blanched, cooled, frozen	18	.17±.007	12	.62±.018[3]
Frozen, stored 1 month	18	.15±.006	12	.54±.025[3]
Frozen, stored 2 months	18	.14±.006[3]	12	.54±.021[3]
Frozen, stored 3 months	17	.14±.009[3]	12	.48±.020[3]
Frozen, stored 4 months	18	.13±.007[3]	12	.47±.020[3]
Raw	18	.18±.009	12	.90±.020
Steam blanched	18	.17±.008	12	.83±.023[3]
Steam blanched, cooled	18	.16±.008	12	.68±.034[3]
Steam blanched, cooled, frozen	18	.16±.007	12	.70±.019[3]
Frozen, stored 1 month	18	.15±.006	12	.59±.019[3]
Frozen, stored 2 months	18	.14±.007[3]	12	.55±.019[3]
Frozen, stored 3 months	18	.14±.007[3]	12	.53±.025[3]
Frozen, stored 4 months	18	.13±.007[3]	12	.53±.020[3]

[1] Standard deviation of the mean $= S\,\overline{X} = \sqrt{\frac{\Sigma x^2}{N\,(N-1)}}$

[2] Significant difference between raw and treated samples. (Difference is considered significant if the difference between the means is three or more times the standard error of the difference.)

Standard error of the difference $= \sqrt{(S\overline{X}_1)^2 + (S\overline{X}_2)^2}$

[3] Significant difference between water-blanched and steam-blanched samples.

The results of the work done in 1943 (Tables 3 and 4) are quite similar to those obtained in the 1942 study. When the hot water-blanched snap beans were analyzed after blanching, blanching and cooling, and quick freezing, they contained 85, 80, and 80 per cent, respectively, of their original ascorbic acid content. The snap beans which had been blanched by steam retained 89, 86, and 84 per cent, respectively. Farrell and Fellers (1942) reported that snap beans lost 33 per cent of their ascorbic acid during the processes prior to freezer-locker storage.

TABLE 3

Ascorbic Acid Content of Snap Beans and Cauliflower as Affected by Home Methods of Hot-Water and Steam Blanching—1943

Treatment	Snap beans			Cauliflower		
		Ascorbic acid			Ascorbic acid	
	Number of determinations	Mean and standard deviation of the mean as determined	Retention on raw-weight basis	Number of determinations	Mean and standard deviation of the mean as determined	Retention on raw-weight basis
		mgm./gm.	*pct.*		*mgm./gm.*	*pct.*
Raw	20	.20±.005	....	16	.81±.032	....
Water blanched	14	.19±.006	85	16	.72±.032[2]	88
Water blanched and cooled	16	.17±.006[1]	80	16	.64±.026[1]	82
Water blanched, cooled, frozen	16	.17±.004[1]	80	16	.64±.027[1]	81
Raw	19	.19±.006	....	16	.81±.028	....
Steam blanched	16	.18±.005	89	15	.87±.021[2]	104
Steam blanched and cooled	16	.17±.004	86	16	.72±.017	90
Steam blanched, cooled, frozen	16	.16±.005[1]	84	16	.67±.026[1]	82

[1] The difference between the means of the raw and treated samples is significant. [2] The difference between the means of the water-blanched and steam-blanched samples is significant.

TABLE 4

Effect of Freezer Storage on Ascorbic Acid Content of Quick-Frozen Snap Beans and Cauliflower Blanched by Hot Water and Steam Methods—1943

Treatment	Snap beans			Cauliflower		
		Ascorbic acid			Ascorbic acid	
	Number of determinations	Mean and standard deviation of the mean	Retention on raw-weight basis	Number of determinations	Mean and standard deviation of the mean	Retention on raw-weight basis
		mgm./gm.	*pct.*		*mgm./gm.*	*pct.*
Blanched by hot water						
Stored 1 month	16	.16±.007[1]	72	16	.61±.025[1]	76
Stored 3 months	16	.13±.006[1]	59	16	.53±.025[1]	66
Stored 6 months	16	.09±.004[1]	39	16	.43±.024[1]	53
Stored 9 months	16	.07±.007[1]	34	16	.36±.028[1]	44
Blanched by steam						
Stored 1 month	16	.14±.006[1]	74	15	.58±.029[1]	72
Stored 3 months	16	.13±.004[1]	66	16	.51±.022[1]	64
Stored 6 months	16	.08±.004[1]	45	16	.40±.027[1]	49
Stored 9 months	16	.06±.004[1]	30	16	.30±.021[1]	36

[1] The difference between the means of the raw and treated samples is significant..

The differences in the ascorbic acid content of the cauliflower blanched by the two methods are not significant except for the values obtained immediately after blanching. In this instance, as in the 1942 study, the steam-blanched cauliflower retained more ascorbic acid during blanching but lost a correspondingly larger amount during the cooling process. When analyzed approximately 20 hours after freezing, the water-blanched cauliflower retained 81 per cent and the steam-blanched cauliflower retained 82 per cent of the original ascorbic acid.

The frozen vegetables showed further losses of ascorbic acid during storage (Table 4). After a storage period of nine months, the ascorbic acid content of snap beans blanched by hot water had decreased from 80 to 34 per cent of the original raw value, and that of steam-blanched beans from 84 to 30 per cent. Jenkins, Tressler, and Fitzgerald (1938) concluded that if the vitamin C is to be conserved, the maximum storage temperature permissible is —18°C.(0°F.) but that in the case of snap beans, spinach, and broccoli a somewhat lower temperature should be used. Very little ascorbic acid was lost from frozen cauliflower and snap beans after six months' storage at —18° C., according to Jenkins, Tressler, Moyer, and McIntosh (1940). In the present study the ascorbic acid content of frozen cauliflower blanched in hot water was reduced from 81 to 44 per cent during the nine months of freezer storage; and that of frozen cauliflower blanched in steam, from 82 to 36 per cent. In the majority of instances, the ascorbic acid values decreased significantly between the third and sixth, and again between the sixth and ninth months of freezer-locker storage.

SUMMARY

The mean values and standard deviations of the means of fresh, raw Stringless Black Valentine snap beans grown in 1942 and 1943 were 0.18 $\pm$ 0.009 and 0.20 $\pm$ 0.005 milligram of ascorbic acid per gram, respectively. Snap beans blanched by the hot-water method lost approximately 15 per cent of their ascorbic acid content during the blanching procedure and 20 per cent during all the processes preparatory to freezer storage. The steam-blanched snap beans lost 11 per cent during blanching and a total of 16 per cent during the preparatory processes.

The mean values and standard deviations of the means of the raw cauliflower purchased in 1942 and 1943 were 0.81 $\pm$ 0.032 and 0.90 $\pm$ 0.020 milligram per gram, respectively. When ready for freezer storage, the cauliflower blanched by hot water was found to have lost 19 per cent of the original ascorbic acid and that blanched by steam had lost 18 per cent.

Both frozen vegetables showed a decrease in ascorbic acid content during storage. After nine months of freezer storage, snap beans and cauliflower had lost approximately 60 to 70 per cent of their original total ascorbic acid.

There were no significant differences in the amounts of ascorbic acid present in quick-frozen snap beans prepared by the hot-water or by the steam method of blanching followed by cooling in running water, nor were any differences found after one, three, six, and nine months of

freezer-locker storage. Similar results were obtained with quick-frozen cauliflower.

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INFLUENCE OF CHEMICAL CHARACTERISTICS OF SOIL ON PRODUCTION OF CAROTENE IN LEAVES OF OATS[1]

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The great value of cereal grasses as sources of protein and vitamins for human food depends on the comparatively high concentrations of these substances which exist in the leaves just before jointing begins. Since all standards of fertility are arbitrarily based on some specific agriculturally important response of crops, it is evident that different aspects of the general problem of the nutrition of plants appear as different aspects of the response of plants are observed. The very extensive literature dealing with the nutrition of cereal plants centers largely around the effects of the nutritional environment on the yield or quality of the grain or on the vegetative yield and quality of the plant when harvested for hay. Comparatively little is known about the effects of these conditions on the protein and vitamin contents of the leaves when harvested at, or just before, the jointing stage.

The chemical characteristics of a group of soils in the vicinity of Midland, Douglas County, Kansas, have been described by Wynd and Romig (1943). Wynd and Noggle (1945a) reported that the total amount of replaceable bases in these soils was their most important chemical property in limiting the production of dry matter of oats harvested at the jointing stage. Calcium was found to be the most important component of the replaceable bases, particularly in the less productive soils. The effect of calcium appeared to be of great importance probably because such a large proportion of the replaceable bases consisted of this nutrient, and not necessarily because other replaceable bases were of less intrinsic importance. The amount of nitrogen in the soils of this area was of secondary importance in controlling the yield of oats harvested at the jointing stage.

Wynd and Noggle (1945b) later reported the influence of the chemical properties of these soils on the accumulation of protein in the leaves of oats. It was found that the amount of nitrogen in the soil was the dominant factor in determining the percentage of protein in the leaves. Since the amount of replaceable magnesium in the soils in the vicinity of Midland is directly proportionate to the nitrogen content in the base-saturated soils, it was not possible to separate the effects of these substances. However, if the soils were not saturated with bases, the amount of nitrogen present appears to be more important than the replaceable bases. This situation makes it appear reasonable to suppose that nitrogen was the more important nutritional factor.

[1] The expenses incurred in the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

The present paper describes the effects of the chemical characteristics of six soils in the vicinity of Midland on accumulation of carotene in the leaves of oats.

EXPERIMENTAL PROCEDURE

The determinations of the carotene content were made on the same samples which furnished the data for the study of growth (1945a) and for the study of the accumulation of protein (1945b). Carotene was determined according to the method described by Buxton and Dombrow (1938) except that methyl alcohol was substituted for ethyl alcohol, and the heptane portion was concentrated on a hot plate in a flask with a vertical condenser instead of by vacuum distillation under nitrogen. One gram of powdered, dehydrated oat leaves was refluxed on a hot plate with 50 milliliters of alcoholic potassium hydroxide. This solution was prepared by dissolving one pound of potassium hydroxide in one liter of methyl alcohol. After cooling, the mixture was transferred by flushing with 100 milliliters of purified heptane into a 500-milliliter separatory funnel. The tissue residue and the alcoholic potassium hydroxide portion were drawn off into another separatory funnel and successively extracted with 50-milliliter portions of heptane until a colorless solution was obtained. Usually four or five extractions were sufficient. The heptane extractions were combined and washed free of chlorophyllins, flavones, xanthophylls, and alkali, by shaking thoroughly with successive 50-milliliter portions of 90 per cent methyl alcohol until the washings were free from alkali. Usually five washings were sufficient. The first alcohol washing was extracted with 50-milliliter portions of heptane and this washing was added to the main portion. The heptane extract was concentrated on a hot plate to about 25 to 30 milliliters, and then quantitatively transferred to a 50-milliliter, glass-stoppered, volumetric flask and brought to volume. A one-milliliter aliquot was placed in a colorimetric tube together with nine milliliters of heptane. The percentage of the light transmitted was measured by an Evelyn photoelectric colorimeter. A No. 420 filter was used. The colorimeter was standardized by a series of known solutions of carotene containing, respectively, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 grams of carotene per milliliter. The readings obtained corresponded to 10, 15, 20, 30, 40, 50, 60, 70, and 80 milligrams of carotene per 100 grams of sample. The values were plotted against the logarithm of the galvanometer readings by means of semilog graphing paper. The resultant standard curve was used to translate the readings obtained from the experimental samples into milligrams of carotene per 100 grams of tissue.

The data (Table 1) are expressed in terms of milligrams of carotene per 100 grams of oven-dry tissue, and also as yields of carotene in pounds per acre. The M.S.C. file numbers refer to the original samples permanently on file in the Department of Botany and Plant Pathology at Michigan State College. The chemical data for each of the soils may be identified in the tables published by Wynd and Romig (1943) by referring to the corresponding file numbers of the soils. The point numbers identify in the figures the soils from which each of the data was obtained.

The soils included in the study may be classified into two groups on the basis of their content of replaceable bases and degree of base satura-

tion. These properties are important in the interpretation of the data presented in the figures. The soils of Fields 2A and 2B (Points 1 and 2) are dark-colored loams which contain comparatively large amounts of replaceable bases, are not calcareous, and have a low pH and a comparatively low degree of base saturation. These soils contain exceptionally large amounts of organic matter as compared with other soils in this area. Their high base-exchange capacity is due to this accumulation of organic matter. For further details, the papers by Wynd and Romig (1943) and Wynd and Noggle (1945a, b) should be consulted.

TABLE 1

Carotene in Leaves of Oats

M. S. C. file No.		Date	Field No.	Point No.	Dry matter per acre	Carotene	
Soil	Oats					Content	Yield per acre
					lb.	*mg./ 100 gm.*	*lb.*
267	315	Oct. 8, 1940	2A	1	221	41.8	.092
268	317	Oct. 11, 1940	2B	2	304	49.8	.151
269	319	Oct. 17, 1940	19	3	200	33.3	.067
272	321	Oct. 21, 1940	12	4	389	45.8	.178
274	318	Oct. 23, 1940	14B	5	253	37.0	.094
280	325	Nov. 1, 1940	18	6	552	44.3	.245

EXPERIMENTAL RESULTS

The base-exchange capacity is shown by Fig. 1A to bear a very definite positive relationship to the concentration of carotene in the leaves of immature oats. The amount of organic matter determines to a very great extent the base-exchange capacity, and also the amount of nitrogen in the soils in the vicinity of Midland. The distribution of the points therefore is almost identical in Figs. 1A, 11A, and 12A. The amount of nitrogen is undoubtedly the significant component of this group of related characteristics of the soil in its effect on the concentration of carotene in the leaves. The base-exchange capacity also is related to the yield of carotene in pounds per acre, especially since this factor bears a positive relationship to the yield of dry matter (1945a) as well as to the percentage of carotene in the tissue. This effect on the yield of carotene is due largely to the amount of replaceable bases which are retained by the colloids in the soil rather than to the nitrogenous material itself which is responsible for the major portion of the base-exchange capacity.

The amount of replaceable bases, as shown by Fig. 2A, appears to be related to the concentration of carotene. It is not probable, however, that this soil factor is directly responsible for this effect. The bases are retained in the soil by nitrogenous organic colloids, and the supply of nitrogen which therefore accompanies the bases is the effective component of this complex group of soil factors Soils represented by Points 3 to 6, inclusive, exhibit an almost quantitative relationship between their contents of replaceable bases and their percentage of nitrogen, and therefore the distributions of these points in Figs. 2A and 12A are identical. On the

other hand, Points 1 and 2 represent soils of high nitrogen content but which contain comparatively low amounts of replaceable bases. Fig. 2A shows these points are irregularly located with respect to the general distribution of the remaining points in the figure, yet these points fall well into line if they are plotted on the basis of their base-exchange capacity in Fig. 1A, the percentage loss on ignition in Fig. 11A, or the percentage nitrogen in the soil in Fig. 12A. A comparison of these figures thus reveals that the nitrogen in the soil is of greater importance than the total amount of replaceable bases in determining the concentration of carotene in oat leaves. The effect of the total amount of replaceable bases is shown by Fig. 2B to be closely related to the yield of carotene. This is due particularly to the effect of the bases on the yield of dry matter.

The degree of base saturation appears in Fig. 3A to be negatively related to the concentration of carotene, especially for soils of low base-exchange capacity. The degree of base saturation may be regarded indirectly as the ratio of the total amount of replaceable bases to the nitrogen in the soil because of the association of these factors as components of the base-exchange complex. Low ratios accompany higher concentrations of carotene in the tissue, a fact which verifies the above statement that the nitrogen in the soil rather than the bases affects the concentration of carotene. Points 1 and 2 represent soils rich in nitrogen, but comparatively poor in bases, and their locations in Fig. 3A are therefore irregular. The effect of the degree of base saturation on the total yield of carotene is especially interesting since it shows that the favorable influence of the total amount of replaceable bases on the yield of dry matter is less important than is the effect of nitrogen on the concentration of carotene in governing the total yield of carotene.

The effect of pH of the soil on the concentration of carotene is difficult to interpret. Usually, the pH of a soil is a reflection of the degree of base saturation of its colloidal surfaces, but a comparison of the distribution of Points 3 to 6, inclusive, in Figs. 3A and 4A, shows that this is not true for this group of soils. Comparison of Figs. 4A and 14A shows that the pH of these soils is related to the amount of calcium carbonate which they contain, although it should be borne in mind that the presence of carbonate in air-dry soil samples may produce pH values in the sample different from those in the fresh soil. The higher amounts of carbonate accompany higher amounts of replaceable bases and also of nitrogenous organic matter. In fact, these increases in organic matter are relatively greater than those of the bases. Apparently, the pH values of these soils, as determined in the laboratory, were affected more by the higher amounts of carbonates which they contained than by their lower degree of base saturation.

The effect of pH on the yield of carotene is even more confusing. In the absence of other data, we would assume an explanation similar to that given above for the effect of pH. However, Wynd and Noggle (1945a) have pointed out that the yield in dry matter is primarily determined by the larger amounts of bases occurring in soils of higher pH, while in the present study it is apparent that the concentration of carotene is governed largely by the increasing amounts of nitrogen present in these soils which have the higher pH values. Even though the explanation of the conditions

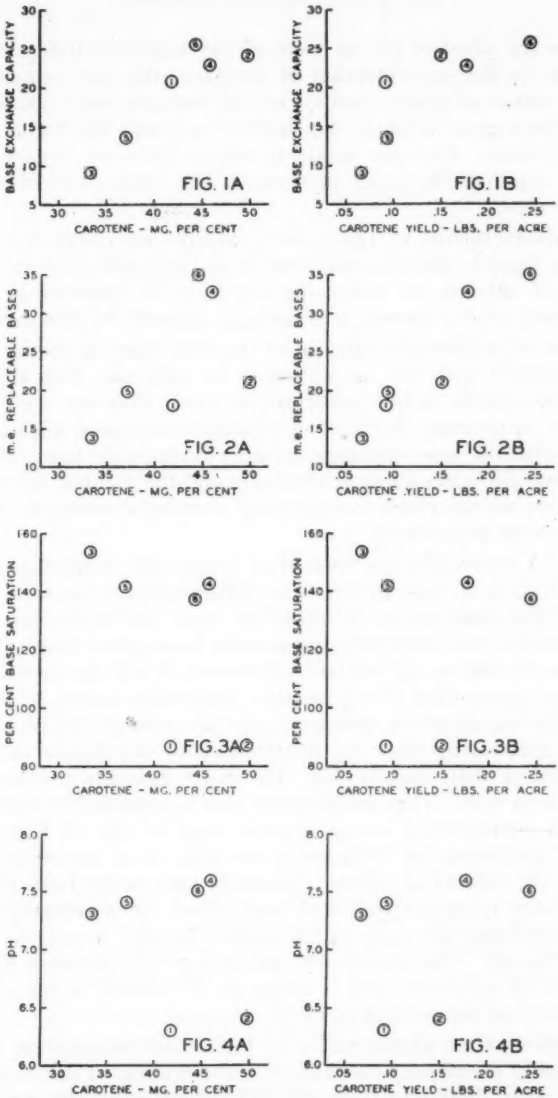

FIG. 1. A, Relationship of base-exchange capacity of soils and concentration of carotene in leaves of oats. B, Relationship of base-exchange capacity and yield of carotene.

FIG. 2. A, Relationship between total amount of replaceable bases in soil and concentration of carotene in leaves of oats. B, Relationship of total amount of replaceable bases and yield of carotene.

FIG. 3. A, Relationship of degree of base saturation of soils and concentration of carotene in leaves of oats. B, Relationship of degree of base saturation to yield of carotene.

FIG. 4. A, Relationship of pH of soils and concentration of carotene in leaves of oats. B, Relationship between pH and yield of carotene.

which cause the effect of pH on yield of dry matter is different from that of its effect on the concentration of carotene, the fact remains that the higher pH values as determined on air-dry samples were definitely associated with the higher yields of dry matter and with the higher concentrations of carotene. The net result in either instance, regardless of the theoretical explanation, is an increase in the total yield of carotene in pounds per acre.

The milliequivalents of replaceable calcium are shown by Fig. 5A to have only a vaguely positive relationship to the concentration of carotene. The effect of calcium on increasing the yield of carotene is very great since the yield of dry matter is so greatly affected by this nutrient.

The ratio of replaceable calcium to the total bases is shown by Fig. 6A to vary inversely with the concentration of carotene. This situation may exist because calcium is less important by itself than the combined effects of all bases, or because of the effect of some component, such as nitrogen, associated with the base exchange capacity-replaceable base complex. The same relationship exists between this ratio and the yield of dry matter, and yield of carotene therefore is negatively correlated with the lower ratios of calcium to the total bases.

Figure 7A shows that the amount of replaceable magnesium in the soil appears to bear a very definite positive relationship to the concentration of carotene. This observation is consistent with the statement above that some component associated with replaceable bases other than calcium influences the accumulation of carotene. However, it will be shown below that the great apparent effect of replaceable magnesium is due to the fact that its concentration parallels quantitatively the amount of nitrogen in the soil. It is noteworthy that the distribution of the points in Fig. 7A is almost identical with that in Fig. 1A which presents the base-exchange values of these soils. This distribution also is remarkably similar to that in Fig. 11A representing loss on ignition and to that in Fig. 12A representing the percentage of nitrogen in the soil. It is impossible, therefore, to separate the individual effects of these factors on the basis of these data since they vary quantitatively with each other. It is remarkable that the amount of exchangeable magnesium should parallel so closely the organic matter in the soil. The replaceable magnesium also appears to be related to the yield of carotene since it seems to be related to the yield of dry matter as well as the concentration of carotene.

The higher values of the ratios of replaceable magnesium to the total bases generally accompany the higher concentration of carotene. There is no definite effect of this ratio on the yield of carotene, although the distribution of the points in Fig. 8B resembles closely that for the yields of dry matter presented in Fig. 8 in the authors' report (1945a) on the yield of dry matter. The fact that low ratios of replaceable magnesium to the total bases in the soil are associated with the higher concentrations of carotene in the leaves suggests that the effect of magnesium alone is greater than that of all bases. This is an absurdity since the amount of magnesium was itself included in the amount of total bases. The explanation of this inconsistency depends on the fact, stated above, that the amounts of

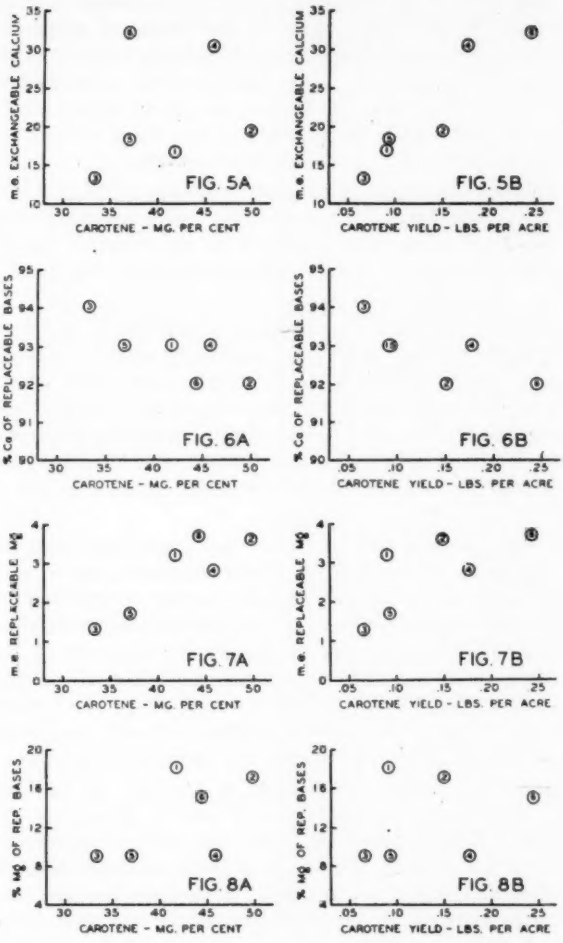

FIG. 5. A, Relationship between amount of replaceable calcium in soils and concentration of carotene in leaves of oats. B, Relationship between amount of replaceable calcium and yield of carotene.

FIG. 6. A, Relationship between magnitude of ratio of replaceable calcium to total replaceable bases in soils and concentration of carotene in leaves of oats. B, Relationship between magnitude of ratio of replaceable calcium to total replaceable bases and yield of carotene.

FIG. 7. A, Relationship between amount of replaceable magnesium in soils and concentration of carotene in leaves of oats. B, Relationship between amount of replaceable magnesium and yield of carotene.

FIG. 8. A, Relationship between magnitude of ratio of replaceable magnesium to total replaceable bases in soil and concentration of carotene in leaves of oats. B, Relationship between magnitude of ratio of replaceable magnesium to total replaceable bases and yield of carotene.

replaceable magnesium parallel the amount of organic nitrogen in the soil, consequently the favorable effect of low ratios of magnesium to the total bases is due to the larger amounts of nitrogen related to the replaceable magnesium in these soils. It is also possible, although highly improbable in view of the data concerning the great influence of nitrogen, that the ratio of replaceable magnesium to the total amount of bases is intrinsically significant to the metabolism of oat plants.

The amount of replaceable potassium is shown by Fig. 9A to bear no relationship to the concentration of carotene. An irregularly positive relationship between potassium and the yield of dry matter causes the yield of carotene also to show a similar vaguely positive relationship to this nutrient.

The ratio of the amount of replaceable potassium to the total replaceable bases is shown by Fig. 10A to be negatively related to the concentration of carotene. This relationship probably is the result of the absence of significant influence of potassium and the highly positive relationship of the nitrogen-magnesium complex. A similar negative relationship exists between this ratio and the yield of dry matter, and therefore the yield of carotene is shown by Fig. 10B to be negatively related to the magnitude of this ratio.

The very close relationship between the percentage loss on ignition and the concentration of carotene has been mentioned above as dependent on the nitrogenous nature of the organic matter in the soil. The effect of the loss on ignition on the yield of dry matter has already been shown by the authors (1945a) to be due to the higher amounts of bases which the organic matter retains in the soil; hence the yield of carotene is indirectly dependent on this factor. Points 1 and 2 (Fig. 11B) represent soils containing a high amount of organic matter but only moderate amounts of bases, and their location in the figure is therefore irregular.

The relationship between the amount of nitrogen in the soil and the concentration of carotene in the leaves of oats is shown by Fig. 12A to be very striking. Figure 12B shows that the yield of carotene is not as closely related to the nitrogen in the soil as is the concentration of carotene. However, if Points 1 and 2 are ignored, the remaining data show a very close relationship between the yield of carotene and soil nitrogen. The data numbered 3, 4, 5, and 6 are from base-saturated soils in which the amounts of replaceable bases and of nitrogen are both limited by the base-exchange complex. Consequently the production of dry matter and the concentration of carotene are both limited by factors which vary concomitantly. The irregular location of Points 1 and 2 is due to the fact that they represent soils which have a high amount of nitrogen which influences the carotene content of the crop, but only a moderate amount of replaceable bases which limits the production of dry matter. This limitation of the production of dry matter causes lower yields of carotene than would be expected otherwise.

The amount of phosphorus in the soil which is soluble in weak acid is shown in Figs. 13A and 13B to bear no relationship to the concentration, or to the yield, of carotene.

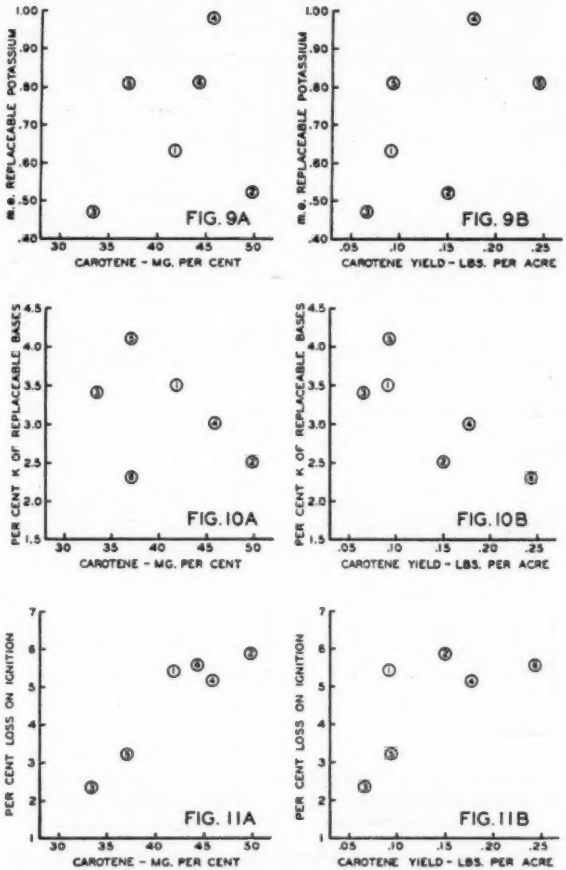

FIG. 9. A, Relationship between amount of replaceable potassium in soils and concentration of carotene in leaves of oats. B, Relationship between amount of replaceable potassium and yield of carotene.

FIG. 10. A, Relationship of magnitude of ratio of replaceable potassium to total replaceable bases in soils and concentration of carotene in leaves of oats. B, Relationship between magnitude of ratio of replaceable potassium to total replaceable bases and yield of carotene.

FIG. 11. A, Relationship between percentage of soils lost on ignition and concentration of carotene in oat leaves. B, Relationship between percentage of soils lost on ignition and yield of carotene.

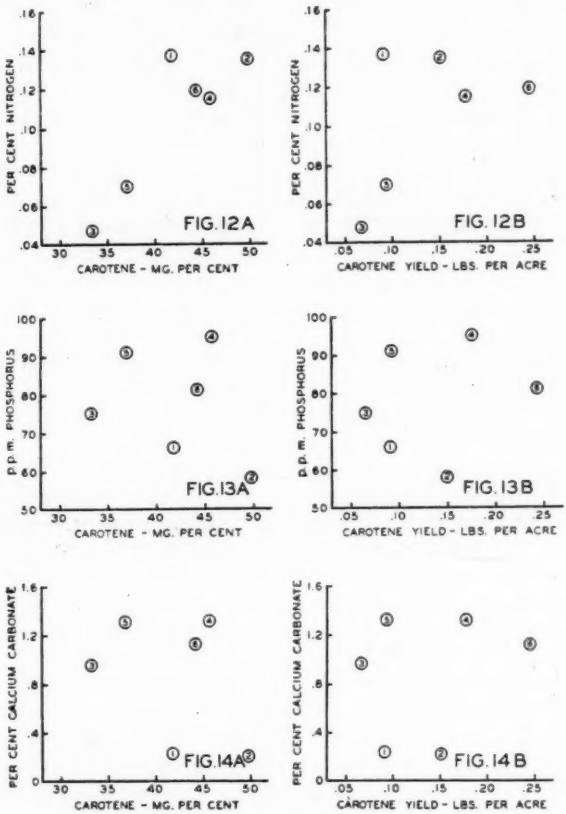

FIG. 12. A, Relationship between percentage of nitrogen in soils and concentration of carotene in leaves of oats. B, Relationship between percentage of nitrogen and yield of carotene.

FIG. 13. A, Relationship between amount of phosphorus in soils soluble in weak acid and concentration of carotene in leaves of oats. B, Relationship between amount of phosphorus soluble in weak acid and yield of carotene.

FIG. 14. A, Relationship between amount of calcium carbonate in soils and concentration of carotene in leaves of oats. B, Relationship between amount of calcium carbonate and yield of carotene.

The relationship of the amount of carbonate in the soil is shown by Fig. 14A to be only slightly, if at all, related to the concentration of carotene. The small degree of relationship which does appear is probably due to the higher amounts of nitrogenous organic matter which accompany the high amounts of carbonate. Soils represented by Points 1 and 2 are not calcareous. The small amounts of carbonate which appear to be present are probably due to the unavoidable evolution of carbon dioxide from organic matter during treatment of the samples with the acid solutions of stannous chloride.

DISCUSSION

A survey of all the data presented in the figures shows that the percentage of nitrogen in soil is probably the most important chemical feature of the soil in determining the concentration of carotene in the leaves of immature oats. Replaceable magnesium appears to be equally important, but the fact that replaceable magnesium varies quantitatively with the nitrogenous organic matter makes it impossible to separate its effect from that of nitrogen.

The effect of soil properties on the yield of carotene in pounds per acre depends on the amount of dry matter produced as well as on the concentration of carotene in the tissue. Since the first depends primarily on the total amount of replaceable bases, while the latter is dependent on the amount of nitrogen in the soil, it follows that the tissue which yields the higher total amounts of carotene need not necessarily contain the highest concentration.

In many instances, the amount of replaceable bases retained in these soils depends on the magnitude of the base-exchange capacity. Since the base-exchange capacity is due largely to nitrogenous organic matter, it follows that some soils produce high yields of carotene in oat leaves because the high amounts of bases produce large yields of dry matter, and the high amounts of nitrogen cause a high concentration of carotene. These soils, which exhibit a high degree of base saturation, produce high yields of dry matter which contain high concentrations of carotene.

Dark-colored soils in the vicinity of Midland contain a large amount of nitrogenous organic matter but only comparatively small amounts of replaceable bases. These soils, which exhibit a low degree of base saturation, produce a high concentration of carotene in oat leaves because of the nitrogen in the soil but comparatively small yields of dry matter because of the low concentration of replaceable bases.

SUMMARY

The accumulation of carotene in the leaves of immature oats grown on six soils in the vicinity of Midland, Douglas County, Kansas, is related to the chemical properties of the upper eight inches of the profile. The amount of nitrogen is the most important chemical property of the soil in controlling the concentration of carotene in the leaves.

The base-exchange capacity and percentage loss on ignition also appear to affect the concentration of carotene since they parallel the amount of nitrogen in the base-saturated soils. The amounts of replaceable calcium and magnesium also appear to be related to the concentration of carotene,

although their individual effects are obscured by the organic nitrogen which accompanies them in the soil.

The amount of replaceable potassium and weak acid-soluble phosphorus appear to have no relationship to the accumulation of carotene in the leaves. The degree of base saturation, the pH value, and the amount of carbonate do not affect directly the accumulation of carotene, although they may appear to be important if variations in their amounts accompany or reflect similar variations in the amounts of replaceable bases or of nitrogen.

Rich soils, exhibiting a high degree of base saturation, may produce large yields of dry matter which contain high concentrations of carotene, because the amount of replaceable bases is the most important factor affecting growth, while the nitrogen is the most important influence on the concentration of carotene. Soils exhibiting a low degree of base saturation may produce small yields of dry matter because of their low content of replaceable bases but a high concentration of carotene because of their comparatively high content of nitrogenous organic matter.

The concentration and yield of carotene appear to be governed by the same soil properties which determine the percentage and yield of protein.

The yield of carotene in pounds per acre depends primarily on the yield of dry matter.

Satisfactory soils for the production of carotene in immature oat leaves in the vicinity of Midland are characterized by soil properties of at least the following magnitudes: base-exchange capacity, 20 milliequivalents per 100 grams of soil; total replaceable bases, 20 milliequivalents; replaceable calcium, 18 milliequivalents; replaceable magnesium, two milliequivalents; loss on ignition, four per cent; and nitrogen, 0.09 per cent. Soils containing less than 15 milliequivalents of replaceable bases or less than 0.08 per cent nitrogen are unsatisfactory for the purpose described.

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INFLUENCE OF CHEMICAL CHARACTERISTICS OF SOIL ON PRODUCTION OF VITAMIN C IN LEAVES OF OATS[1]

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The chemical properties of the surface soils of a group of soil sites in the vicinity of Midland, Douglas County, Kansas, have been described by Wynd and Romig (1943). Wynd and Noggle (1945a) have described the relationships between these properties and the growth of oats, the accumulation of protein (1945b), and of carotene (1945c) in the leaves of oats. The amount of replaceable bases, especially of calcium and magnesium, was found to be the dominant chemical characteristic of these soils in determining the yield of dry matter when the crop was harvested at the jointing stage. The percentage of crude protein and the milligrams of carotene per 100 grams of tissue depended primarily on the nitrogen content of the soil. The total yields in pounds per acre of protein, as well as of carotene, were governed by the yields of dry matter rather than by the concentration of these substances in the tissue.

The vitamin contents of plant tissue vary within wide limits, even under conditions of growth which appear to be entirely normal. Reference to the tables of the vitamin contents of foods, published in current textbooks on human nutrition, unfortunately reveals that these variations are usually ignored. It is tacitly assumed that the vitamin content is determined by the kind or variety of plant, although the variations in different samples of the same species or variety of vegetable tissue grown under different conditions may exhibit a range of variation in vitamin content as great as that between different species and varieties.

The concentration of vitamin C in plant tissues is particularly variable; for example, unpublished observations of the authors have shown that its concentration in fresh leaves of cereal plants may vary from 75 to 1,100 milligrams per 100 grams when calculated on the basis of the oven-dry tissue. So little is known concerning the causes of these variations that additional information should be of interest not only to the plant physiologist, but to students of human nutrition as well. The present study extends the authors' previously published observations to include the influence of soil characteristics on the vitamin C accumulation in the leaves of oats. The extensive use of the leaves of immature cereals as a source of vitamins for human consumption lends additional importance to the study of the relationship between the nutritional condition of these species and their vitamin contents.

EXPERIMENTAL PROCEDURE

The six soils included in the present study may be classified into groups on the basis of their content of replaceable bases and their degree

[1] The expenses incurred in the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

of base saturation. Fields 2A and 2B (Points 1 and 2 in the figures) are dark-colored loams which contain comparatively large amounts of replaceable bases, are not calcareous, and have a low pH and a low degree of base saturation. They contain large amounts of organic matter as compared with other soils in the vicinity, and their high base-exchange capacity and nitrogen content is due primarily to the organic matter. The soils of Fields 19, 12, 14B, and 21 (Points 3 to 6, inclusive) are brown clay loams. They exhibit a high pH and a high degree of base saturation. They differ from each other primarily in the amount of replaceable bases which they contain. It is essential that these major differences be borne in mind during the study of the data presented in the figures. For further details concerning these soils, the papers by Wynd and Romig (1943) should be consulted.

The determinations of vitamin C were made on the same samples which furnished the data for the previous reports on the effects of soil properties on growth (1945a), on the percentage and yield of protein (1945b), and on the concentration and yield of carotene (1945c). The crop was harvested at the jointing stage and dried in an Arnold hot-air dehydrator. The vitamin C was determined immediately by triturating a one-gram sample in a mortar with 25 milliliters of an extracting solution containing two per cent of metaphosphoric acid and three per cent of trichloroacetic acid. The addition of two to three grams of purified Ottawa sand hastened the disintegration of the tissue and permitted a more reproducible extraction to be made. The mixture was filtered through Reeve Angel No. 202 filter paper. One milliliter of the filtrate was titrated with a standard dye solution. Glycerin was used to lubricate the stopcock of the microburette from which the dye solution was delivered. The dye solution was prepared by dissolving 1.5 grams of the sodium salt of 2,3-dichlorophenolindophenol in one liter of dioxane which contained one per cent of acetic acid. The dioxane was freshly distilled just before it was used in preparing the dye solution. The resultant dye solution was standardized against one milliliter of a freshly prepared solution of ascorbic acid, made by dissolving 10 milligrams of ascorbic acid in 50 milliliters of an acid mixture containing two per cent of metaphosphoric acid and three per cent of trichloroacetic acid. The concentration of vitamin C in the sample was calculated as follows:

$$\frac{\text{ml. of dye solution} \times 100{,}000}{\text{dye factor}} = \text{mg. per cent vitamin C}$$

where the dye factor represented 200 times the milliliters of the dye solution which reacts with 0.2 milligram of ascorbic acid.

EXPERIMENTAL RESULTS

The base-exchange capacity of the soil is definitely related to the concentration of vitamin C in the leaves of immature oats (Fig. 1A). Organic matter in these soils largely determines their base-exchange capacity and nitrogen content, and therefore the distribution of the points in Fig. 1A is very similar to that in Fig. 11A which relates the percentage loss on ignition to the vitamin C content of the crop, and also to the distribution

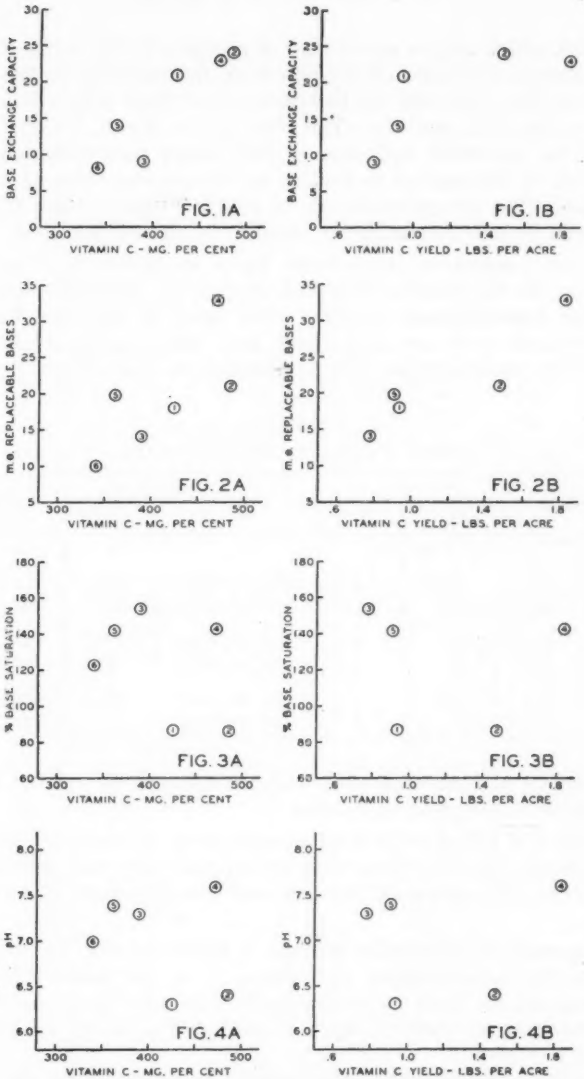

FIG. 1. A, Relationship between base-exchange capacity of soils and concentration of vitamin C in leaves of oats. B, Relationship between base-exchange capacity and yield of vitamin C.

FIG. 2. A, Relationship between amount of replaceable bases in soils and concentration of vitamin C in leaves of oats. B, Relationship between amount of replaceable bases and yield of vitamin C.

FIG. 3. A, Relationship between degree of base saturation of soils and concentration of vitamin C in leaves of oats. B, Relationship between degree of base saturation and yield of vitamin C.

FIG. 4. A, Relationship between pH value of soils and concentration of vitamin C in leaves of oats. B, Relationship between pH value of soils and yield of vitamin C.

in Fig. 12A which relates percentage of nitrogen in the soil to the vitamin C in the leaves. The effect of the base-exchange capacity on the total yield is shown in Fig. 1B, and the distribution of these points is also similar to that in Figs. 11B and 12B. The effect on the yield of vitamin C is due partly to the favorable influence of the higher base-exchange capacities on the yield of dry matter as well as on the concentration of the vitamin in the tissue. The irregular location of Points 1 and 2 (Figs. 1B, 11B, and 12B) should be noted carefully in relation to discussions below.

The total amounts of replaceable bases are shown by Fig. 2A to be related also to the concentration of vitamin C, although less definitely than is the base-exchange capacity. The effect of the total bases on the yield of vitamin C is very great since they affect the yield of dry matter as well as the concentration of this vitamin to a vast extent.

TABLE 1

Vitamin C (Ascorbic Acid) in Oat Leaves

U. of I. file No.		Field No.	Point No.	Date	Dry matter per acre	Vitamin C	Vitamin C per acre
Soil	Oats						
					lb.	*mg./ 100 gm.*	*lb.*
267	315	2A	1	Oct. 8, 1940	221	425	0.939
268	317	2B	2	Oct. 11, 1940	304	486	1.477
269	319	19	3	Oct. 17, 1940	200	389	0.778
272	321	12	4	Oct. 21, 1940	389	472	1.836
274	318	14B	5	Oct. 23, 1940	253	361	0.913
273	323	21	6	Oct. 31, 1940	.....	341	.....

The degree of base saturation does not seem to be related to the concentration or to the yield of vitamin C.

The effect of pH of the soil on concentration of vitamin C in the leaves is presented in Fig. 4A. There does not appear to be any definite relationship between pH values of the soil and concentration of vitamin C in the crop.

The amount of replaceable calcium is shown by Fig. 5A to be directly related to the concentration of vitamin C in the tissue. The effect of the calcium on the total yield of vitamin C is very great since it is positively related to the yield of dry matter as well as to the concentration of vitamin C.

The relationship between the magnitude of the ratio of replaceable calcium to the total replaceable bases and the concentration of vitamin C in the leaves of oats is presented (Fig. 6A); the relationship is seen to be indefinite. If Point 6 is disregarded, then small ratios are associated with the high concentration of vitamin C, which suggests that the effect of calcium alone is less than that of the total bases. The favorable effect of the smaller ratios on the total yield is apparent from a study of Fig. 6B.

Higher amounts of replaceable magnesium accompany higher concentrations of vitamin C (Fig. 7A). The arrangement of the points in Fig. 7A is almost identical with their arrangement in Fig. 11A, which presents the

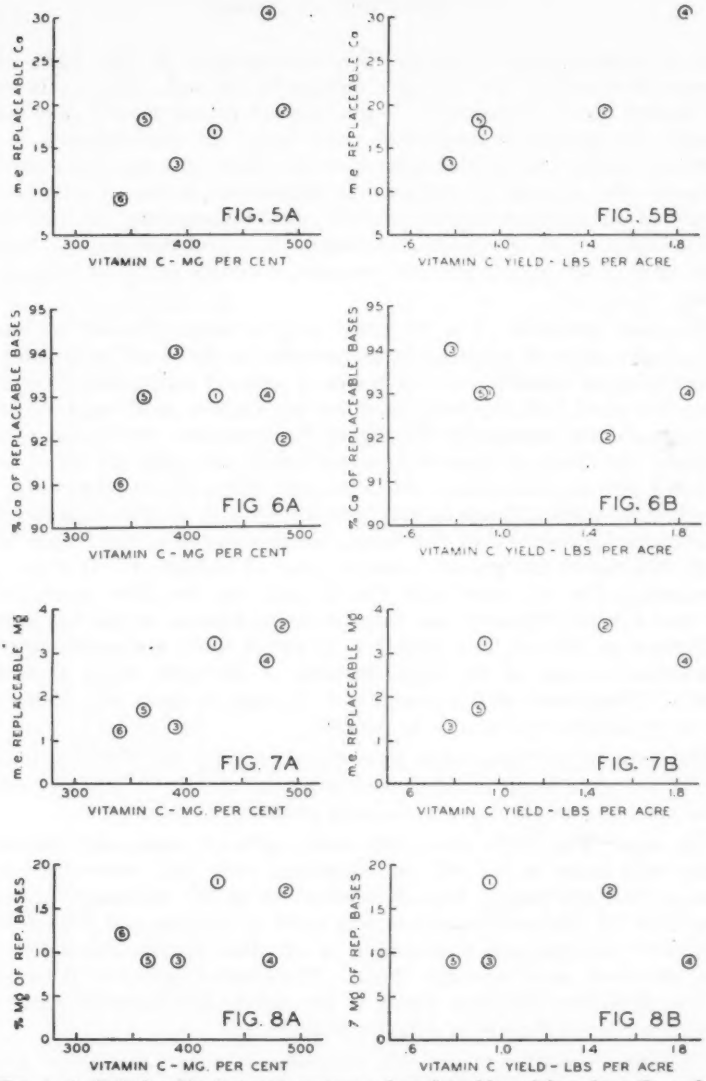
m e REPLACEABLE Ca
VITAMIN C - MG. PER CENT
FIG. 5A
m e REPLACEABLE Ca
VITAMIN C YIELD - LBS PER ACRE
FIG. 5B
% Ca OF REPLACEABLE BASES
VITAMIN C - MG. PER CENT
FIG 6A
% Ca OF REPLACEABLE BASES
VITAMIN C YIELD - LBS PER ACRE
FIG. 6B
m e REPLACEABLE Mg
VITAMIN C - MG. PER CENT
FIG. 7A
m e REPLACEABLE Mg
VITAMIN C YIELD - LBS PER ACRE
FIG. 7B
% Mg OF REP. BASES
VITAMIN C - MG. PER CENT
FIG. 8A
% Mg OF REP. BASES
VITAMIN C YIELD - LBS PER ACRE
FIG 8B

FIG. 5. A, Relationship between amount of replaceable calcium in soils and concentration of vitamin C in leaves of oats. B, Relationship between amount of replaceable calcium and yield of vitamin C.

FIG. 6. A, Relationship between magnitude of ratio of replaceable calcium to total replaceable bases in soils and the concentration of vitamin C in leaves of oats. B, Relationship between magnitude of replaceable calcium to total replaceable bases and yield of vitamin C.

FIG. 7. A, Relationship between amount of replaceable magnesium in soils and concentration of vitamin C in leaves of oats. B, Relationship between amount of replaceable magnesium and yield of vitamin C.

FIG. 8. A, Relationship between magnitude of ratio of replaceable magnesium to total replaceable bases in solids and concentration of vitamin C in leaves of oats. B, Relationship between magnitude of ratio of replaceable magnesium to replaceable bases and yield of vitamin C.

effect of organic matter, and to their arrangement in Fig. 12A, which presents the effect of the nitrogen content of the soil. Figure 7B shows that higher yields of vitamin C also appear to accompany the higher amounts of replaceable magnesium, and again the arrangements of the points are similar to those which show the effect of organic matter and nitrogen. The amount of replaceable magnesium varies directly as the amount of nitrogenous organic matter, and consequently its individual effect is obscured by the effect of nitrogen. It is probable that the amount of nitrogen is the most important component of this group of related soil factors.

The data presented (Fig. 8A) show only a vague effect of the magnitude of the ratio of replaceable magnesium to the total bases and the concentration of vitamin C in the leaves of oats. If magnesium were influencing the plant independently of other soil factors, small magnitudes of this ratio should accompany the higher concentration of vitamin C since obviously the effect of magnesium alone could not equal the effect of all the bases. On the other hand, the combined effects of the magnesium and the nitrogen which appears to vary directly with it, could be greater than the combined effects of all the bases. In this instance, the larger ratios would accompany the greater concentration of vitamin C. If Point 4 is disregarded, Fig. 8A shows that this is true. On the other hand, Points 3, 5, and 6 could represent low concentrations because of the low amount of nitrogen in the soil, and Points 1, 2, and 4 could represent high concentrations because of the high amounts of nitrogen which these soils contain. Comparison of the amounts of nitrogen in these soils shows that this latter interpretation also is possible.

The amount of replaceable potassium is shown by Fig. 9A to have little or no relationship to the concentration of vitamin C. Its relationship to the yield of vitamin C is a little more evident.

The data (Fig. 10A) show that small ratios of replaceable potassium to the total bases in the soil are associated with high concentrations of vitamin C in the tissue. This is verification of the statement presented above that the effect of potassium was small or lacking and the effects of replaceable calcium and magnesium, or of other factors associated with these nutrients, were especially great. The smaller values of these ratios are associated also with high yields of dry matter and therefore their relationship to the total yield of vitamin C is evident.

The organic matter in the soil, represented by the percentage loss on ignition, is shown by Fig. 11A to be markedly related to the concentration of vitamin C. The positive relationship between the organic matter and the yield of vitamin C is very evident since this soil component is also positively related to the yield of dry matter.

The higher amounts of nitrogen in the soil are shown by Fig. 12A to bear a direct relationship to the concentrations of vitamin C in the tissue of oat leaves. There is also a positive relationship to the total yield of vitamin C because the yield of dry matter, as well as the concentration of the vitamin, is influenced. It has already been stated that the similarity of the observed relationship of the base-exchange capacity and the organic

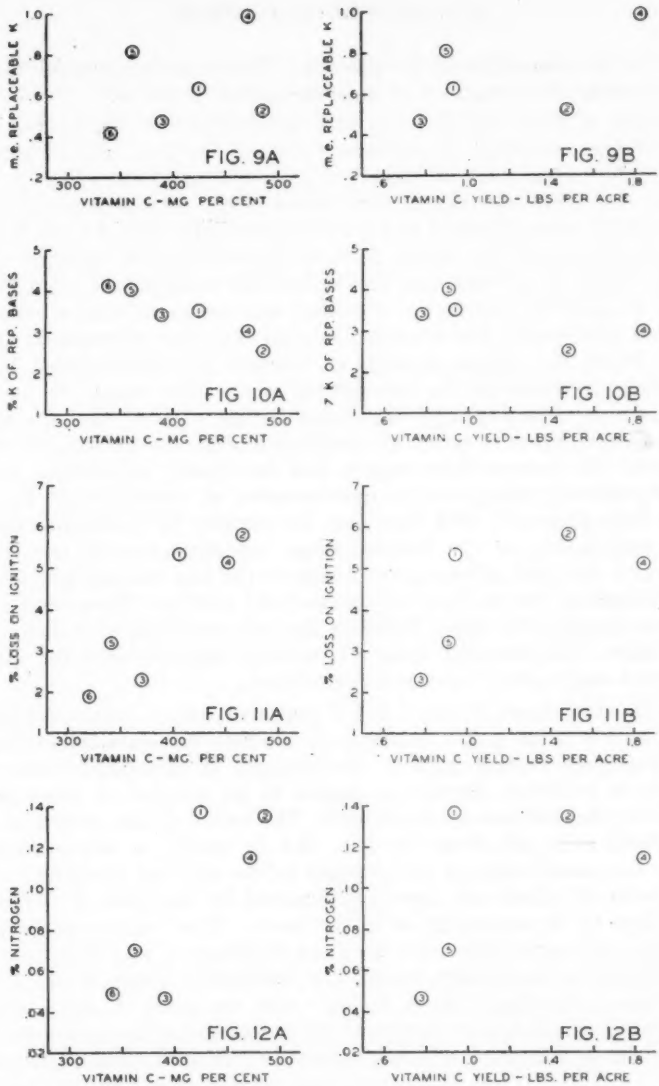
m.e. REPLACEABLE K
VITAMIN C - MG PER CENT
FIG. 9A
m.e. REPLACEABLE K
VITAMIN C YIELD - LBS PER ACRE
FIG. 9B
% K OF REP. BASES
VITAMIN C - MG PER CENT
FIG 10A
% K OF REP. BASES
VITAMIN C YIELD - LBS PER ACRE
FIG 10B
% LOSS ON IGNITION
VITAMIN C - MG PER CENT
FIG. 11A
% LOSS ON IGNITION
VITAMIN C YIELD - LBS PER ACRE
FIG 11B
% NITROGEN
VITAMIN C - MG PER CENT
FIG. 12A
% NITROGEN
VITAMIN C YIELD - LBS. PER ACRE
FIG. 12B

FIG. 9. A, Relationship between amount of replaceable potassium in soils to concentration of vitamin C in leaves of oats. B, Relationship between amount of replaceable potassium and yield of vitamin C.

FIG. 10. A, Relationship between magnitude of ratio of replaceable potassium to total replaceable bases in soils and concentration of vitamin C in leaves of oats. B, Relationship between magnitude of ratio of replaceable potassium to total replaceable bases and yield of vitamin C.

FIG. 11. A, Relationship between percentage of soil lost in ignition and concentration of vitamin C in leaves of oats. B, Relationship between percentage of soil lost in ignition and yield of vitamin C.

FIG. 12. A, Relationship between amount of nitrogen in soils and concentration of vitamin C in leaves of oats. B, Relationship between amount of nitrogen and yield of vitamin C.

matter to the concentration of vitamin C is due to nitrogenous material which governs the magnitude of these properties of the soil.

A study of Figs. 1B, 11B, and 12B, which show the relationship of the base-exchange capacity, of percentage loss on ignition, and of nitrogen, respectively, to the yield of vitamin C, reveals that Points 3 to 5, inclusive, are distributed according to an almost linear relationship to the yield of vitamin C, while Points 1 and 2 are eccentrically located in each figure. The distribution of the points in these figures furnish evidence of the relative effects of nitrogen and the replaceable bases on the yield of vitamin C. Points 3 to 5, inclusive, represent soils whose colloidal surfaces are saturated with bases. The colloidal material is largely nitrogenous organic matter, hence the higher amounts of nitrogen are accompanied by corresponding increases in the amounts of replaceable bases. It has been shown in a previous paper by Wynd and Noggle (1945a) that the amount of replaceable bases is positively correlated with the growth of oats in these soils. The present data suggest that the amount of nitrogen in these soils is positively related to the concentration of vitamin C in the crop. Hence, soils saturated with bases, in the vicinity of Midland, exhibit a direct relationship of the base-exchange capacity, organic matter, and nitrogen to the yield of vitamin C, owing to the fact that greater amounts of bases increase the yields of dry matter and that the nitrogenous matter which accompanies the bases increases the concentrations of vitamin C. It is not known how generally these relationships are true since the data are based on a single set of nutritional conditions.

On the other hand, Points 1 and 2 represent soils in which the amount of replaceable bases is not sufficient to saturate the colloidal surfaces of the nitrogenous organic matter. The amount of nitrogen in these soils therefore is relatively greater in respect to the amount of bases present than is the case in base-saturated soils. The leaves of oats grown in these unsaturated soils are shown by Fig. 12A to contain a concentration of vitamin C proportionate to the nitrogen in the soil, but the total yield of the vitamin in pounds per acre is determined by the yield of dry matter rather than by its concentration in the leaves. This is consistent with the data (Fig. 2A) which show that the yield of vitamin C was directly related to the amount of replaceable bases. The locations of Points 1 and 2 in the three figures mentioned above indicate that the yield of dry matter in unsaturated soils is less in respect to the amount of nitrogen present than it would be in saturated soils, and therefore the total yield of vitamin C is less.

Figure 13A shows a negative relationship between the amounts of phosphorus soluble in weak acid in the soil and the concentration of vitamin C in the tissue, if Point 4 is disregarded, although it is not certain that the elimination of this point is justifiable. A similar relationship of phosphorus to the yield of vitamin C is shown by Fig. 13B.

The amount of carbonate in the soil is shown by Fig. 14A to have no intrinsic effect on the concentration of vitamin C. Soils represented by Points 1, 2, and 6 are noncalcareous. Any significance that might be ascribed to the amount of carbonate in soils represented by Points 3, 4,

and 5 probably would be due to the amount of replaceable bases or of organic matter which are also present.

A survey of the data presented in all the figures shows that the relationships of the chemical properties of the soils to the vitamin C in the leaves of oats are not as easily recognized as their relationship to the yield of dry matter (1945a) and to the production of protein (1945b) and carotene (1945c) reported in previous papers. It does appear, however, that the soils which contain the larger amounts of replaceable bases and nitrogen produce the greater concentration and yield of vitamin C. It is

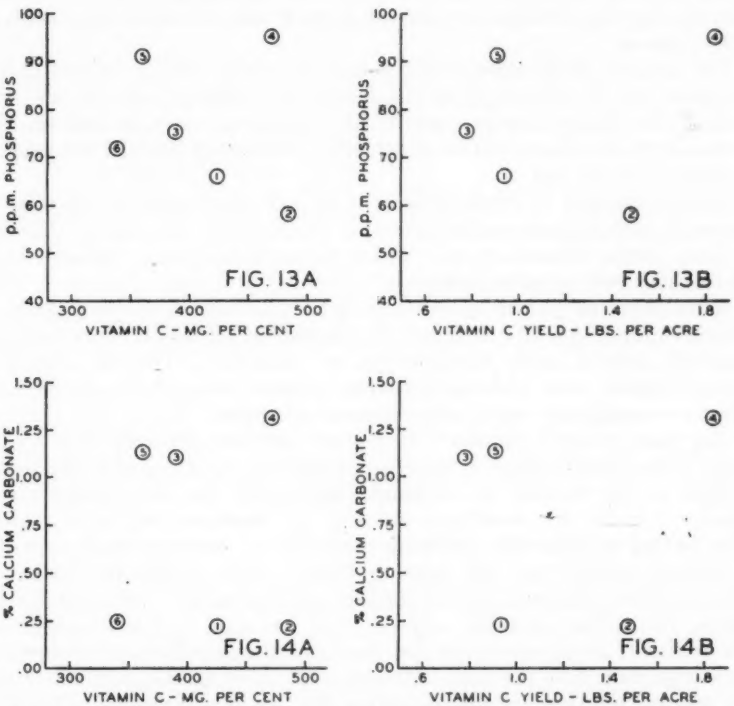

FIG. 13. A, Relationship between amount of phosphorus in soil, soluble in weak acid, and concentration of vitamin C in leaves of oats. B, Relationship between amount of phosphorus soluble in weak acid and yield of vitamin C.

FIG. 14. A, Relationship between amount of calcium carbonate in soils and concentration of vitamin C in leaves of oats. B, Relationship between amount of calcium carbonate and yield of vitamin C.

probable that the amount of nitrogen is the most important chemical characteristic of the soil in governing the concentration of vitamin C, at least under the conditions under which these plants were grown. Irrespective of the effect of individual factors of the soil, it is clear that the plants which grow the most rapidly and give the higher yields of dry matter when harvested at the jointing stage, contain the greater concentration of vita-

min C. The total yield of vitamin C in pounds per acre is always governed by the yield of dry matter.

SUMMARY

The chemical properties of six soils in the vicinity of Midland, Douglas County, Kansas, were studied in relation to their effects on the accumulation of vitamin C in the leaves of oats harvested at the jointing stage.

The relationships of soil properties to the vitamin C content of oat leaves are not as evident as their relationships to the growth and the production of protein and carotene reported previously by the authors. Plants which grow rapidly and produce the higher yields of dry matter, generally but not always, contain the greater concentration of vitamin C in their leaves.

The amount of nitrogen in the soil is especially clearly related to the concentration of vitamin C in the leaves of cereals grown on the soils studied. The base-exchange capacity and percentage loss on ignition also are related to the concentration of vitamin C since they parallel the amount of nitrogen in the soil.

Higher amounts of replaceable calcium and magnesium in the soil are associated with higher concentrations of vitamin C in the leaves although the nitrogenous organic matter which accompanies them obscures their individual effects in some instances.

Replaceable potassium shows little or no relationship to the vitamin C content of the crop. The amounts of phosphorus soluble in weak acid are negatively related to the concentration of vitamin C. The pH, degree of base saturation, and carbonate content appear important only if they reflect or accompany replaceable bases or nitrogen.

The total yield of vitamin C in pounds per acre depends on the yield of dry matter rather than on the concentration of the vitamin in the tissue.

Soils in the vicinity of Midland, satisfactory for the production of vitamin C under the conditions studied, are characterized by soil properties having at least the following magnitudes: base-exchange capacity, 15 milliequivalents per 100 grams of soil; total replaceable bases, 20 milliequivalents; replaceable calcium, 18 milliequivalents; replaceable magnesium, two milliequivalents; nitrogen, 0.09 per cent; and loss on ignition, four per cent. Soils containing less than 15 milliequivalents of replaceable bases, less than 0.07 per cent nitrogen, and showing less than three per cent loss on ignition are unsatisfactory for the production of vitamin C in oat leaves under the conditions studied.

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